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ERRATA

Page 41, paragraph 2, line 3, *read* Wakker *for* Walker.

Page 84, paragraph 1, line 1, *before* unshaded *insert* in.

Page 110, line 2, *change* rye, barley *to* those mentioned above and.

Page 219, table title and footnote 2, *read* Juglans *for* Jugulans.

Page 231, paragraph 3, line 3, and paragraph 4, line 7, *read* diethyldithiocarbamate *for* diethyldithiocarbonate.

Page 342, paragraph 2, line 7, *after* as *insert* by.

Page 472, series 37, and page 496, line 6 of text, *change* Kolotex *to* Kolodust.

Page 559, line 22, *change canavaline to canavaliae.*

Page 600, paragraph 3, line 2, *change* acropetal necrosis *to* stipplestreak.

Page 708, cuadro 2, *insert* heading, Trigos a 14 pares de cromosomas, for four last-named varieties, coordinate with heading, Trigos a 21 pares de cromosomas.

Page 714, line 7 from bottom, *read* "población" *for* "popblación."

Page 716, paragraph 5, line 1, *read* variabilidad *for* variabilidod.

Page 718, paragraph 2, line 2, *read* extremadamente *for* extremandamente.

Page 721, line 17 from bottom, *read* notar *for* notas; *read* trabajo *for* trabojo; line 3 from bottom, *substitute* Lineas susceptibles a la par que otras muy resistentes, se encontraron *for* en trigos "poblaciónes" tales como el Fulcaster, Gipsy, Harvest.

Page 722, paragraph 1, line 10, *read erythrospermum for esythrospermum.*

Page 964, paragraph 1, line 2, *read Champini for Chantini.*

Page 980, figure legend, line 5, *read* beat- *for* heat-.

Page 1133, table 1, column heading 4, *read* Number infected *for* Number inoculated.

Outside cover page, December, Contents, line 14; page 1181, title, paragraph 1, line 3, and paragraph 2, line 1; page 1183, paragraph 2, line 1, and paragraph 4, line 2; *change* malvaceara *to* malvacearum.

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An International Journal

Official Organ of
The American Phytopathological Society

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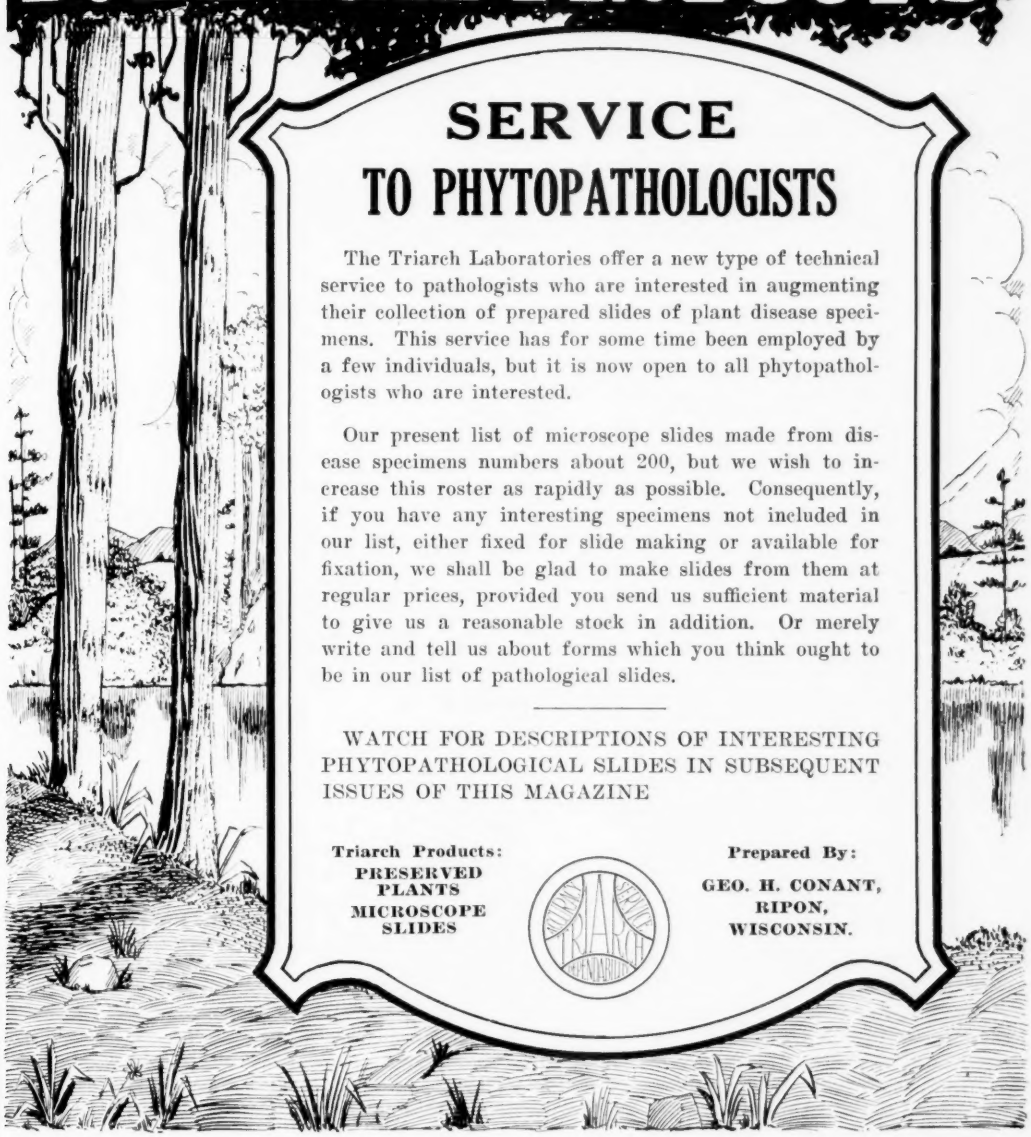

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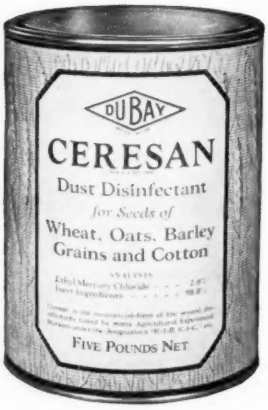

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PHYTOPATHOLOGY

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JANUARY, 1931

WILLIAM ALLEN ORTON
1877–1930

LEWIS RALPH JONES

No loss from the ranks of American plant pathology has ever been personally mourned more widely and sincerely than that of Dr. W. A. Orton. Few had more varied friendships than his in Washington's scientific and administrative circles or more wide-reaching in those of the United States. Beyond our national confines his personal relations and professional correspondence encircled the globe. He was at once an internationalist of world-wide vision and interest and a friendly adviser to everyone who sought his aid. His breadth of interest and charm of personal qualities made him the helpful counselor for a host of foreign scientific visitors who made Washington the point of entry to American agricultural institutions. He was himself an experienced traveler and by skillful planning of itineraries and courteous letters of introduction he contributed much to the happiness and success of many such foreign guests.

Dr. Orton exemplified in a peculiar way the heroic in science. To those who knew his ancestry and early development this was not surprising. He came from the best Vermont stocks. As his name suggests, the blood of the Allens flowed in his veins. Since the days of "The Green Mountain Boys," this has stood for dauntless leadership, forgetful of personal dangers. The name of Orton also has long been known in New England science and administration as well as in pulpit and school. Born in a Vermont village, North Fairfax, he entered the Agricultural course in the University of Vermont when he was only sixteen and received his baccalaureate degree with honors (Phi Beta Kappa) at twenty. This was the better evidence of his native genius because from his childhood he was handicapped by partial deafness. As his botany teacher I well recall my impressions of this immature freshman always in the front seat as I lectured, eyes open, lips parted, eagerly "drinking in" every idea, although many of the words must have escaped him. I do not recall that he took a note but he always understood and retained. To the last weeks of life he showed remarkable powers of concentration upon any question under discussion, with unfailing ability to analyze and hold the essentials. In his student days, as in his later life

with its greater physical trials, instead of yielding to handicaps he forced them to contribute to his successes. If he recognized any limitations it was only that he might turn from lesser tasks to concentrate the more effectively upon the greater ones. Upon these he never admitted the possibility of failure.

For two years after graduation he remained at the University as an advanced student and research assistant. His chief interests concerned the parasitic fungi of Vermont, reviewing the literature, reworking all available material, collecting eagerly, and listing for publication. One of his University classmates of this period recalls a day when "Mr. Orton, an eager youth of somewhat indifferent college preparation, came into the laboratory saying that he had to use Latin books on mycology (Saccardo) although he had never studied Latin. To the admiration and chagrin of his fellow students who had spent several years upon Latin, young Orton acquired in a surprisingly short time a practical use of the language." He assisted also in our experimental work with potato diseases, inaugurating interests which guided him into important developments a decade later.

In 1899, when I was a guest in the laboratory of Dr. Erwin F. Smith in Washington, he told of his need of a promising research man for work on the control of cotton wilt. Immediately I urged consideration of young Orton, and he accepted my judgment. It certainly was something of a venture to accept for such a task a half-trained boy of twenty-two who had never even seen a cotton plant. With such brief suggestions as could be given in Washington, Orton went alone to spend the summer with the Carolina planters. He afterwards confessed his initial lonesome homesickness. But here, as always, his quick vision of the bigness of the job made him oblivious to all petty hindrances. His kindly personality, keen perception, sterling integrity, and tireless devotion to his tasks won the confidence and enduring friendship of the Carolina planters. From them he soon learned whatever their experience had taught them about cotton. Doctor Smith, following Atkinson's early work, had already diagnosed the cotton wilt as a soil-borne, vascular Fusarium disease. Seed disinfection and spraying were thus useless. Experiments with soil fungicides and fertilizers gave negative results. Hygienic measures, sanitation, and rotation, suggested by Smith, were only palliatives, pronounced impractical by the planters. What was to be done? Fortunately Orton had entered the old Division of Vegetable Physiology and Pathology, later merged into the Bureau of Plant Industry, at a time when the work was dominated by a small group of men with whom associations were most stimulating to thoughts along new lines. These included, in addition to Doctors Galloway, Woods, and Smith, such men as Carleton, Fairchild, Swingle, Waite, and Webber. Under such in-

fluences increasing attention was being given to the physiological aspects of phytopathological problems along with genetics and disease resistance in relation to control measures. Carleton had just brought from northern and eastern Europe his first large collection of foreign cereals as a basis for breeding for resistance to rust. Webber and Swingle were diffusing through the departmental ranks the glowing enthusiasm for plant improvements recently kindled by their epochal work in Florida on frost-resistant citrus. Orton was an eager young listener at such discussions. Almost immediately, as he surveyed the cotton fields of Carolina, he saw the possibilities of wilt control through disease resistance. Within two years he had the most remarkable evidence produced to that date as to the practical significance of disease resistance in plant-disease control. His first departmental publication, in 1900, "Wilt Disease of Cotton and Its Control," will remain a historic classic. This recorded the result of selections for disease resistance made the first summer and brought to completion after but two summers' work in the southern cotton fields by this inexperienced Vermont youth in his early twenties. Yet his conclusions are valid to-day. Along with these studies he had succeeded, where both Atkinson and Smith had earlier failed, in establishing, by pure-culture inoculation, the pathogenicity of this Fusarium on cotton. Nor was this all. His inspiration carried him on to the discovery of the wilt-resistant cowpea, which he, in association with Webber, showed to be the more remarkable in that it was also immune from the root-knot nematode. From this he turned to water-melon wilt, to overcome which he hybridized the resistant citron with the edible melon. These, with Bolley's work on flax, laid the secure foundation for all subsequent work with selection and breeding for resistance to the group of vascular Fusarium diseases. Thus in two years' time he mapped the way to the solution of problems which Smith had in 1898 defined as among the most seriously threatening in the field of phytopathology.[1]

The happy culmination of these earlier observations and researches upon disease resistance came with the formulation of his concepts as to the explanation and classification of disease-resistant types. These were outlined in the Yearbook of the Department of Agriculture for 1908 and in his address before the Conférence Internationale de Génétique, Paris, 1911.

His early work on these wilt diseases in the southern cotton and truck fields represents his outstanding personal researches. In these, except for brief association with Doctor Webber on cowpea breeding, he worked alone. Orton's genius was, however, always stimulated by contacts, and his natural instincts sought companionship.

[1] Smith, Erwin F. The fungus infestation of agricultural soils in the United States. Sci. Am. Supplement 48: 19981. 1899.

His unusual originality in ideas, coupled with his ability in organization for their advancement, was soon recognized in the Department of Agriculture. In 1907 he was made head of the newly created Office of Cotton, Truck, and Forage Crop Disease Investigations. Here his infectious enthusiasm found ampler field. He built up a scientific staff of some forty members, characterized at once by scientific ability, integrity in service, and devoted personal loyalty to their leader. He was a member of the committee of three which guided the organization of The American Phytopathological Society in 1908–09.[2] Of this Society he was a charter member and one of the drafters and signers of the articles of incorporation and held numerous offices, including the presidency and the editorship of PHYTOPATHOLOGY. At the first meeting of the Society it appointed a committee of five, of which he was a member, "to draft resolutions concerning wart disease of potato and white pine blister rust and to take steps to secure such action as would prevent their further introduction and spread." These two serious European diseases had recently appeared on the American Continent in quick succession in 1908 and 1909. From this, with correlated action by the American Association of Economic Entomologists, came the initiation of the Federal plant-quarantine service. The administration of this was placed in the hands of the Federal Horticultural Board, of which he was vice-chairman and pathologist for twelve years. These responsibilities stimulated his breadth of interest and information, especially concerning international problems. He was always a believer in the importance of international cooperation in quarantine matters. Whereas quarantines may naturally tend to create international frictions and material misunderstandings, he saw in them the opportunity for the very opposite. Might they not rather offer foci for international conferences and coordinated researches? To accomplish these he led in the movement, unfortunately interrupted by the World War, to encourage the visits to America of European pathologists and to favor like foreign journeyings of Americans for surveys and studies. The continuing benefits to international fellowship in scientific endeavor are to-day as clearly recognized in European countries as in America and Orton's early services in this field as fully appreciated.

[2] In addition to his official relations with The American Phytopathological Society he was also a charter member of the American Horticultural Society and held membership in other scientific organizations as follows: The American Association for the Advancement of Science, the Botanical Society of America, the Botanical Society of Washington, the Washington Academy of Sciences, the Society for Horticultural Science, the American Society of Agronomy, the American Forestry Association, the American Genetics Association, the American Society of Naturalists, the International Society of Soil Science, the Société de Pathologie Végétal, the Society of American Foresters, the Cuban Association of Sugar Cane Technologists, the International Society of Sugar Cane Technologists, the World Agricultural Society, the Cosmos Club, and Phi Beta Kappa.

His interest in potato wart and in the virus diseases of potato, together with the Conférence International de Génétique, took Orton to Europe in 1911. His earlier interest in potato pathology was thereby enhanced. His contacts with the work of Doctors Appel and Wollenweber in Germany were especially stimulating. Several important developments resulted. The system of potato-seed inspection and certification already in early operation in Germany under Appel's leadership was launched in America, where it continues as one of the great constructive preventive measures. Orton came back convinced of the importance of the virus problems in potato pathology. Through his publications and personal leadership he started the investigations concerning these problems which have ever since continued to increase in recognized scientific complexity and economic importance.

He brought back with him from Germany, as an addition to his staff, Dr. H. W. Wollenweber as Fusarium expert. This was done in order to put American studies of pathogenic Fusarium species into line with those of Europe, a type of contribution of continuing need with like complex problems. Later, he brought also Dr. Otto Appel from Germany that America might benefit from his experience with potato diseases in Germany. He contributed his influence in an important degree to the later visits of other leading European mycologists and pathologists, especially Butler, Cotton, Pethybridge, and Brierley, from Great Britain, Quanjer from Holland, Pole-Evans from South Africa, Foëx from France, and Vavilov and Jaczewski from Russia.

Dr. Orton organized and for several years carried on the plant-disease survey of the Bureau of Plant Industry, which has become of great importance in connection with work on the control of crop diseases. During the World War he was active in organizing research on diseases causing losses of vegetables in transit, market, and storage, which was developed in close cooperation with the food-products inspection service of the Bureau of Agricultural Economics and very materially increased knowledge of the causes of losses in shipment and marketing. As a result of this work methods for the reduction of disease losses in the process of marketing have been developed.

Following the war's interruptions of European international relations, Dr. Orton's vision sensed the increasing need for leadership in agricultural and especially phytopathological problems in the Latin Americas. The organization of our National Research Council gave him occasion for crystallization of these ideas. For nearly a decade, until his death, he was chairman of the Research Council's Committee on Phytopathology in the Tropics. Much thought was given to the correlation of tropical education

and research. Constructive reports were made on graduate education in tropical agriculture. The outstanding specific accomplishment was the organization under the National Research Council of the Tropical Plant Research Foundation. In 1924 he resigned from the Department of Agriculture to become the Scientific Director of this Foundation. This was designed as an agency to provide for tropical countries, particularly in the Western Hemisphere, a scientific service in support of crop production. As Director he gave concrete form to his ideals of such service through science in the interests of progress in two continents already closely interdependent. He always considered these undertakings a logical part of an inclusive national development. It is of interest to record that his most intimate early associates in inaugurating and advancing this project were Dr. George R. Lyman, then Secretary of The American Phytopathological Society, and Fred C. Meier, the present Secretary.

His first thoughts in specific researches dealt with the needs of large tropical-plant corporations such as the United Fruit Company. Such corporations engaged in any phase of plant culture in the Latin Americas are continually in need of reliable expert scientific advice upon pathological or other cultural problems. Their efforts at securing this by the temporary employment of individual expert advisers were, in general, expensive and relatively ineffective. He conceived the possibility of a non-profit-sharing organization under the patronage of the National Research Council, with expert scientific guidance, so functioning as to serve as an intermediary or activating agency in bringing the best scientific service to bear upon the peculiar problems involved.

His leadership of the Tropical Plant Research Foundation as Scientific Director continued until his death. This Foundation rendered expert services to such great corporate interests as the Cuba Sugar Club, representing the united sugar-cane growers of the Island, for which it organized and directed an experiment station. It served in an advisory capacity to various governments of Central and South America upon agricultural problems, including the organization and manning of both experiment stations and agricultural institutions. During the later years Doctor Orton was official agricultural adviser to the Pan-American Union.

In the field of tropical forestry Dr. Orton was a pioneer, giving it an impetus and a standing that it had never before enjoyed in the New World. His early grasp of the fact that in the American tropics the products of the forest are crops whose wise care and harvesting are as necessary as in agricultural crops led him to take a leading part in the little-known field of tropical forestry. An acceptance of silviculture and of conservative logging by the Latin-American Republics he conceived to be a part of his con-

tribution to their agricultural and economic stability. In this field he directed forest work in a number of Latin-American countries, and under his supervision the first survey of tropical American forest resources was made.

It must suffice to comment on only one other specific example, as outlined by Colonel George P. Ahern, formerly Director of the Philippine Forest Service, a Trustee of the Foundation representing forestry: "Dr. Orton at the invitation of the Brazilian Government in 1928 went to that country and discussed their forest problems with the officials. Before he left Brazil he submitted to them a draft of a forest policy so sound and comprehensive that it is marveled at by leading foresters, who are amazed that a man not a technically trained forester could prepare such a splendid document. This will, I believe, serve as a guide to the administration of the new forest service in Brazil for many years to come."

Much of this service was rendered gratuitously, and Doctor Orton in the best sense functioned as a missionary of Agricultural Science. Many expressions of appreciation of this followed Doctor Orton's death. One of these may be quoted from the *Revista de Agricultura de Puerto Rico* published in February, 1930, under the caption "*In Memoriam*:" "Porto Rico counted him among her practical benefactors. The cyclone of September, 1928, found him in our Island. After estimating the extent of the damage to our agriculture occasioned by this great disaster, Doctor Orton organized the work of rehabilitation, prepared reports for the Red Cross, and interested the Federal authorities in the defense of our agriculture, giving Porto Rico the benefit of the dynamic ability which always distinguished him."

All of this was done under increasing personal physical disabilities because of diabetes, which, added to his deafness, would have early inhibited a less courageous soul. Fortunately, these things never dimmed his intellectual vigor. It was chiefly a fight against malnutrition. Here again he forced his very disabilities to serve his scientific ends. Before the discovery of insulin he brought together for experimental culture in his own garden what was undoubtedly the most complete collection ever assembled of foliaceous food plants suited to a diabetic dietary. He mastered the physiological pathology of diabetes as well as the chemistry, culture, and cooking of these plants to a point where he was recognized as the national authority on the culture and use of diabetic foods of this class.[3] With the later use of insulin supplementing these dietary precautions and the wonderful co-

[3] Following Dr. Orton's death the eminent specialist on diabetes, Dr. Elliot P. Joslin, of Boston, wrote as follows: "When in my practice the outlook for diabetes was darkest, Doctor Orton brought the first rays of light. . . . From the very start his interest in diabetes was not confined to himself. He always tried to help some one else, and you know what a stimulus his garden, with its ninety-nine different diabetic vegetables, gave

operation of his wife and family, he fought his way back from the brink of bodily starvation to the amazing efficiency of the later years of his life.

For years his American friends, staff associates, and visiting agricultural scientists from all parts of the world found his home a welcome, grateful place of gathering. The memories will long remain with many of evening walks among his beloved dahlias and the inspiration that followed the free informal fellowship with men from many lands. Of these associations so pleasantly formed, not a few were later destined to continue as significant and productive friendships among students in kindred fields. One must number this great, unfailing gift of friendship in the long list of his contributions to mankind.

Any evaluation of his scientific accomplishments fails except as one recognizes the dominance of a great courageous spirit and a clear intellect over physical handicaps. It was this that made him so influential with and so beloved by all his immediate associates. As one of them writes: "He stood on an eminence apparently without realizing his own greatness. He was so friendly, so tolerant, so just, so modest with all, that it seems to me now we worked with a great man without then understanding his true greatness or the rare privilege of serving with him."

These words may well serve as a closing tribute to William Allen Orton, one of our heroes in science.

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PHYTOMONAS BETICOLA[1]

HARRY A. ELCOCK

INTRODUCTION

This paper presents the results of experiments carried on at the Michigan State College and at Rocky Ford, Colorado, from 1926 to 1928, inclusive, upon the root disease which has been called sugar-beet tuberculosis and which recently Brown (1) has called the bacterial-pocket disease. The investigations here presented deal with the nature and etiology of the disease and the effect of the infection upon the metabolism of the sugar beet. They include also studies on the reaction and affinites of the causal organism as well as observations and experiments to throw light upon the saprophytic existence of the organism in the soil.

The bacterial-pocket disease (*Phytomonas beticola* (Smith, Brown and Townsend) S. A. B.) is known only from the United States, and the reports of its occurrences are limited. The first material was received by the Pathological Laboratory, Bureau of Plant Industry, from Holly and Rocky Ford, Colorado, and Garden City, Kansas. Material used by Dr. Townsend came from Girard, Kansas. In 1923 it was found at Arlington Farm, Rosslyn, Virginia, by Brown (1). The disease was collected in 1926 by G. H. Coons and Dewey Stewart from a field at Lamar, Colorado, in which some hail injury had occurred. It was estimated that at least 10 per cent of the beets in certain portions of the field were affected. Since that time numerous collections have been made at Rocky Ford, Colorado, and other places in the Arkansas Valley, so that it can safely be said that the distribution of the disease is fairly general in this area. Mr. Asa C. Maxson, of the Great Western Sugar Company, has found the disease at Lowell, Wyoming. Extensive search has failed to disclose its occurrence in Michigan, although a number of cases of crown gall have been observed.

It seems then that the distribution of the bacterial-pocket disease is chiefly restricted to the western beet area, the single exception being at Rosslyn, Virginia. It also seems safe to predict that additional observations will show the disease more extensively distributed in the western territory than the few collections would indicate.

The occurrence of "galled" beets has been noted for many years, and these beets have always attracted attention at harvest time. Townsend (11)

[1] Acknowledgment is made to Michigan State College; Office of Sugar Plants, Bureau of Plant Industry; and to the South Dakota State College for facilities furnished for carrying on the work here reported. The writer wishes to acknowledge indebtedness to Dr. G. H. Coons for advice during the course of the work and for help in preparation of the manuscript.

states that when galls begin to appear on the beets in a given field they are at first few in number, increasing from year to year if beets are continued in that field. Speaking of overgrowths of the sugar beet in general, he further states that this type of disease of the sugar beet has increased rapidly in recent years and that it is still on the increase.

From the meager data at present available as to distribution of the bacterial-pocket disease of sugar beet as distinct from crown gall, it is obvious that no determination of actual loss caused by this disease can be made. The studies made in certain fields in Colorado indicate the potentialities of the bacterial-pocket disease. Surveys in various fields at Rocky Ford and Lamar, Colorado, have shown an average incidence of 3 to 4 per cent, while in one case in several large areas of the field, 10 per cent of the beets were affected. It is doubtful if the percentage of galled beets of the tubercle type in the general factory run approaches 1 per cent, but the high percentage found in certain fields indicates that under certain conditions the disease may become of increasing importance.

Losses from the bacterial-pocket disease of beets involve the following considerations: (a) Affected beets show marked tendency to rot (Fig. 1, C). The bacterial-pocket disease does not itself cause extensive necrosis of the affected sugar beet, but the rotting which occurs is mainly attributable to secondary invaders, chiefly saprophytic fungi. The ruptured epidermis and the more or less disintegrated nature of the gall tissue afford easy avenues of entrance for such organisms and, as a result, there is considerable decay of affected beets in storage piles. (b) The bacterial-pocket disease of the beet, as will be obvious from the photographs, causes loss because of the wasteful topping of diseased beets. Since the crowns contain such a high percentage of salts which interfere with the recovery of sucrose in the process of manufacture, the present topping practices necessitate removal of the crown just below the scars of the lowest whorl of leaves. The overgrowths of the bacterial-pocket disease are often on the crown and extend into the main part of the root (Fig. 1, A and B). This means a very large portion of the beet is cut off in the topping process, thus reducing the tonnage yield. (c) The bacterial-pocket disease of the beet, however, produces its losses very largely because of the profound upset of the sugar-beet metabolism which it causes, with the consequent reduction in sugar percentage and purity values. Townsend (11) called attention to the injurious effects that overgrowths produce upon the quality of the sugar-beet roots. He showed that the galls themselves are low in purity and are detrimental in the milling process. From his work it is not clear which type of gall was under observation.

FIG. 1.—The bacterial-pocket canker of sugar beets caused by *Phytomonas beticola*. A and B. Typical overgrowths showing ridges which terminate in galls. C. Decay of older overgrowths. D. Bacterial-pocket cankers located at an injury made in cultivation. E. Gall on garden beet produced by inoculation with *Phytomonas beticola*.

EXPERIMENTAL WORK

In order to determine the extent of sugar percentage reduction and the effect upon the purity coefficient of sugar beets induced by the attack of *Phytomonas beticola*, diseased material collected in the field at Rocky Ford,

TABLE 1.—*Comparative analyses for sucrose and apparent purity of normal beets and of beets affected with the bacterial-pocket disease. Analyses made when beets were approximately two-thirds grown*

Condition of beets	Date 1927	Weight in pounds	Sugar in juice	Coefficient of apparent purity[a]	Size of overgrowths[b]
1. Galled beet	Sept. 15	2¼	11.2	65.5	Small
Normal[c] "		2¼	12.8	77.7	"
2. Galled "	" 15	3¼	10.8		"
Normal "		3¾	12.0		"
3. Galled "	" 21	6	11.4		"
Normal "		4½	13.0		"
4. Galled "	" 25	2¼	10.2	68.0	"
Normal "		2½	13.6	78.4	"
5. Galled "	" 25	2¾	11.2		"
Normal "		3	11.1		"
6. Galled "	" 25	3	11.0		"
Normal "		4	12.6		"
7. Galled "	" 25	2½	11.0		"
Normal "		3¼	11.2		"
8. Galled "	" 15	2	10.8	63.0	Medium
Normal "		2¼	14.8	78.9	"
9. Galled "	" 15	3	10.4	64.5	"
Normal "		2½	13.0	78.3	"
10. Galled "	" 30	2½	10.4	66.2	"
Normal "		3	11.3	78.3	"
11. Galled "	" 25	3¼	11.2		"
Normal "		3¼	11.8		"
12. Galled "	" 15	4¼	9.4		Large
Normal "		3¾	13.0		"
13. Galled "	" 21	4	10.0	65.2	"
Normal "					
14. Galled "	" 21	2½	9.2		"
Normal "		2½	12.4		"
15. Galled "	" 30	2	11.6	72.0	"
Normal "		4¼	11.6	78.0	"
16. Galled "	" 25	4½	9.8	64.0	"
Normal "		3¾	12.3	79.3	"
17. Galled "	" 25	4	10.3		"
Normal "		4½	13.6		"
18. Galled "	" 25	6	9.2		"
Normal "		4½	10.8		"
19. Galled "	" 25	2½	10.6		"
Normal "		2½	13.2		"
20. Galled "	" 15	6	10.6	61.2	"
Normal "		4½	13.0	81.9	"

[a] Computed from solids in beet juice as determined by refractometer.

[b] Overgrowth in each case definitely determined to be due to *Phytomonas beticola*.

[c] The normal beet used as a check was adjacent to the galled one, except where a considerable difference in size made it desirable to choose some other beet within a radius of approximately 4 feet to serve as a check.

Colorado, was analyzed for sugar and purity. The results of these analyses were compared with parallel results obtained from normal beets which stood nearby in the row. The samples were taken from a field of commercial beets of a high sugar strain which were being sampled at weekly intervals for the purpose of determination of the course of sugar production during the season. Whenever a natural gall was found, a beet of approximately the same size was chosen from an area within a radius of 4 feet for a comparative test. The beets were analyzed by the usual individual-beet method (9) and before marked wilting had taken place. (Table 1.)

The sugar content and coefficient of apparent purity are the factors which determine the value of beets for sugar-making purposes. A considerable percentage of the total weight of the beets consists of soluble solids, of which sugar forms the largest portion. The galled beets show a very much lower coefficient of apparent purity due to the larger amounts of impurities in the form of soluble solids other than sugar. The greater the proportion of other soluble solids present the lower the purity, and from such beets it is more difficult to extract the sugar. These diseased beets with their low sugar and apparent purity percentage give syrup from which recovery of crystallizable sugar is poor, and the handling of such beets in the factory is unprofitable.

TABLE 2.—*Sugar percentages of diseased sugar beets contrasted with percentages found in normal beets. Tested at Rocky Ford, Colorado. Summer, 1927*

Variety	Sucrose in juice		Deviation from sucrose percentage of average check	Size of gall[a]
	Beets with overgrowth	Adjacent checks		
	12.5%	14.60%	−2.10	Small
50165–166	11.3	12.53	−1.23	"
	11.5	12.03	− .53	"
	11.0	12.69	−1.69	"
4088–23	10.0	10.84	− .84	Medium
	9.5	10.00	− .50	"
	10.0	10.53	− .53	"
	9.6	10.83	−1.23	"
	11.1	11.80	− .70	"
F. F.–24	9.8	12.82	−3.02	Large
	10.2	12.64	−2.44	"
	8.8	11.51	−2.71	"
	10.0	12.23	−2.23	"
	9.5	11.90	−2.40	"

[a] Gall in each case determined as due to *Phytomonas beticola.*

Another lot of beets was analyzed to furnish additional data on sugar-content relations. A number of artificially inoculated beets from plots of three different varieties were used. These beets were inoculated at intervals, so that on August 29, 1927, when the sugar determinations were made, there were three sizes of gall present, *i.e.*, small, medium, and large. The checks were, in the majority of cases, the two adjacent beets; however, if these did not furnish fair samples because of size, the checks were taken within a 4-foot radius. Attempt was made to choose the normal and diseased beets of as nearly the same size as possible.

In the data given in table 2, the figure for the diseased beet is the actual beet-sugar percentage found, and the check value is the average of the sucrose percentages of the two adjacent normal beets.

The analyses given in tables 1 and 2 are consistent in showing the marked reduction of quality of sugar beets brought about by the bacterial-pocket disease. Even in the cases where the overgrowths were small, there was a lower percentage of sugar.

SYMPTOMS

External characteristics: There are, as Smith, Brown, and Townsend (10) have pointed out, two distinct types of overgrowth occurring on the sugar-beet roots, and these have been designated as "tumors and tuberculosis" (11). The terms crown gall and tubercle or bacterial pocket seem preferable to designate the two types. In the field it is easy to confuse these types of overgrowth unless certain characteristics of the older galls are taken into consideration. In the bacterial-pocket disease of beets the young tubercle is a smooth, yellowish white proliferation that shows no sign of outer-cell disintegration. At this stage, the bacterial-pocket overgrowth might be confused with crown gall if internal structure were not taken into consideration (3). The older overgrowths in the bacterial-pocket disease are characterized by their roughness and strongly fissured surfaces. The diseased root generally shows a tendency to be conical or turnip-shape. That is, the beet does not develop with the normal, long, tapering root, but is short and very broad at the crown. Also, because of a marked excessive bud development, profusion of foliage may accompany this type of growth. The leaves at times come out in groups over the entire crown and often extend down to the ground line. Along with the flattening out of the crown, there generally arise swellings which extend as ridges from about the middle of the root up to the crown. These ridges generally terminate in a large rough overgrowth (Fig. 1, A, B, C.). The primary tubercular focus which is the cause of this ridge development is generally deep within the tissue of the root with secondary tubercles near the apex of the ridges. These aspects are in marked contrast to the condition in other beet-root galls, which gen-

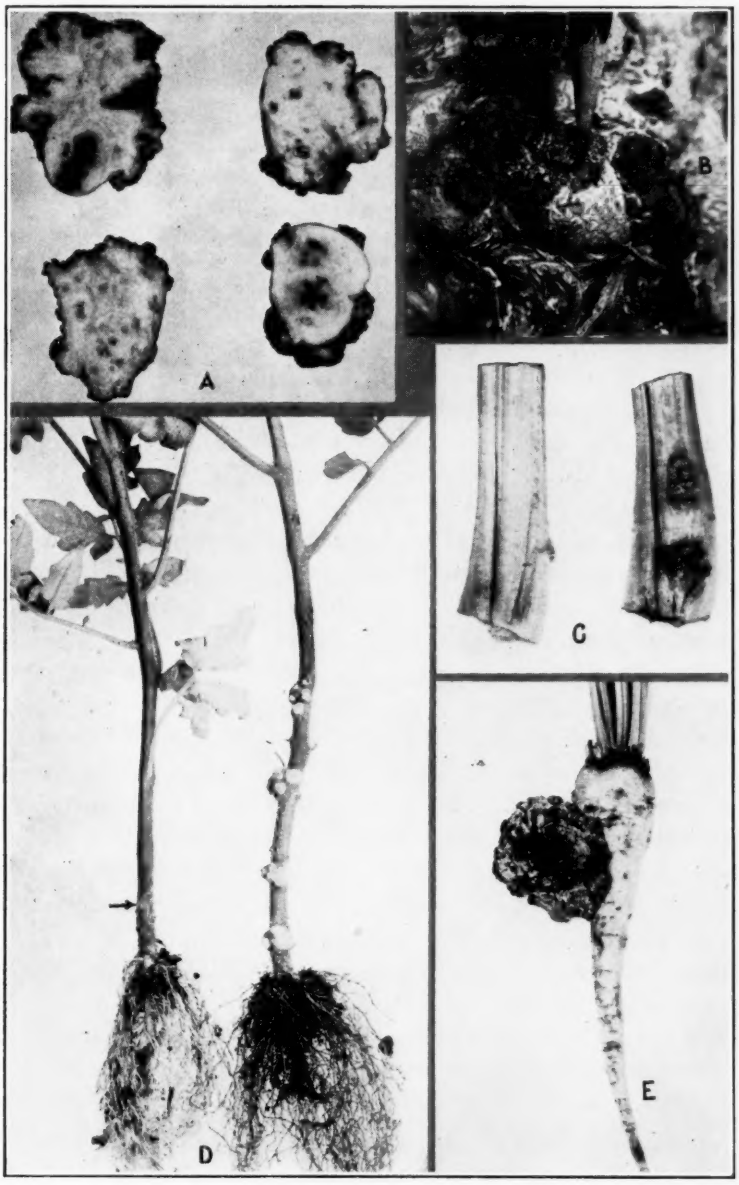

FIG. 2.—A. Bacterial foci characteristic of the pocket canker. B. Mass of bacteria exuding from a gall caused by *Phytomonas beticola*. (Photo by D. Stewart.) C. Galls produced by inoculation of sugar-beet petioles with *Phytomonas beticola*. (Wounded check on left.) D. Tomato plants inoculated with *Phytomonas beticola* (left) and *P. tumefaciens* (right). E. Crown gall of sugar beet caused by *Phytomonas tumefaciens*.

erally are lateral fleshy outgrowths upon the affected roots. In the case of the bacterial-pocket disease, secondary tubercles can be noticed on the surface of the primary tubercle, and these nodular growths, together with the deep fissuring, give the proliferation a very rough, warty appearance. Near the end of the growing season the tubercles show more or less decay of the outer layers of cells. The large fissures and cracks may penetrate the root tissue to a depth of an inch. These openings offer excellent avenues for entrance of secondary fungi, and these, under ordinary conditions, lead to partial decay of the crown (Fig. 1, C.).

Internal characteristics: The internal appearance of this disease is very distinct from that of crown gall. In the bacterial-pocket disease, localized, brownish, watery pockets occur (Fig. 2, A). These bacterial foci give point to the significance and applicability of the name tuberculosis, first applied to the disease. The gall tissues are a result of both hyperplastic and hypertrophic development. Microscopic examination of the brownish, watery spots or pockets, shows large masses of bacteria. These bacterial pockets vary from 2 mm. to 10 mm. in diameter and extend up and down the gall 1 mm. up to 3 to 4 cm. They are filled with material of a viscid consistency, which, under moist conditions, is sometimes so very mucilaginous that the bacterial slime when touched may be drawn out in sticky threads (Fig. 2, B). The diameter of the galls examined varied from about 1 cm. in young infections to as large as 1 dm. in old beets.

Petiole galls: Galls caused by *Phytomonas beticola* on leaf petioles were produced by artificial inoculations of the petioles but have not been seen under natural conditions. The proliferations on the petioles are at first small and appear to come from under the epidermis at the point of inoculation. As the gall becomes older, the epidermis begins to crack and small fissures are formed. The overgrowth then takes much the same rough appearance as the galls on the roots. The galls when young are green, but as the epidermis is ruptured the outer cells disintegrate, and become dry and brown (Fig. 2, C).

Since crown gall and the bacterial-pocket disease of the sugar beet are apt to be confused in reports on sugar-beet galls, the following table is given, which will enable fairly safe field diagnosis to be made. (Cf. Fig. 1, A, B, C, D, and Fig. 2, E.)

Relation of wounds to disease incidence: From field observations made in 1926 and 1927, in Colorado, it appears that the incidence of the bacterial-pocket disease in young, rapidly growing beets is strongly influenced by wounding, provided temperature and water relations are favorable for infection. The disease has been found associated with the wounding of beets

TABLE 3.—*Comparison of bacterial-pocket disease and crown gall of sugar beets*

Bacterial-pocket disease	Crown gall
A bacterial disease caused by *Phytomonas beticola.*	A bacterial disease caused by *Phytomonas tumefaciens.*
Comparison of Overgrowths	
1. Rough, warty, or tubercular overgrowth. Roughness due to rupturing of epidermis. Large fissures develop in the overgrowth.	1. Smooth or wrinkled overgrowth. Contour uneven in old galls, but epidermis usually intact without fissure formation.
2. Portion of root below the galls frequently ridged, ridges extending from middle of root to crown, terminating in the galled outgrowths.	2. Ridges absent; gall generally separated from root by stipe-like growth.
3. Misshapen, ridged, turnip-shape beet roots generally result from this disease, usually with a large amount of newly formed leaves coming out over entire crown.	3. Shape of root usually not changed except at place of tumor formation; no extra growth of leaves.
4. Secondary fungous invasion of the beet commonly prevalent.	4. No secondary fungous growth generally associated with this type of overgrowth.
5. In small, newly developed galls the contour may be uniform, the epidermis intact (later becoming cracked), flesh yellowish white, with numerous yellowish pockets.	5. In small newly formed galls, contour even, epidermis intact, ordinarily does not become fissured, flesh white.
Internal Symptoms of Roots	
1. Numerous yellowish, watery spots present within galls. (Bacterial pockets.) Surrounding tissue, white.	1. No bacterial pockets present; beet tissue, white.
2. Strands not very noticeable, outer cells of overgrowth decayed, fissures extending into tissue.	2. Strands of tissue extending in all directions within gall; outer cells, not decayed.

during cultural operations (Fig. 1, D). For example, mechanical injuries made by a cultivator, just before irrigation, in the case of a field where the disease had been prevalent the previous year led to infection of nearly 60 per cent of the wounded beets. Mr. A. W. Skuderna,[2] of the American Beet Sugar Company, has reported an instance in which an extremely high percentage of bacterial-pocket disease occurred following use of a heavy roller on a field when the beets were in the seedling stage. Crowns of beets often are injured by hail and such injuries have been observed to lead to abundant disease production in fields in southeastern Colorado. The hail storms generally are accompanied by heavy splashing rains which serve to carry the organism to the wounds as well as to provide favorable conditions for establishment of the pathogene. Mr. Asa C. Maxson, of the Great

[2] Verbal communication.

Western Sugar Company, Longmont, Colorado, has made similar observations: "Several times during the past 18 years I have found this type of growth very common in fields badly injured by hail.[3]

The following results of field inoculations made by the writer at Rocky Ford, Colorado, during the summer of 1927, are pertinent to the situations described. Broth cultures of three strains of *Phytomonas beticola* and one strain of *P. tumefaciens* were applied to exposed portions of the sugar and garden beets. In certain cases the inoculated areas were scarified by a few needle scratches, and in other cases the beets were left unwounded. Frequent examinations were made during the first 10 days, and 20 days after inoculation the beets were pulled and final readings taken (Table 4). In

TABLE 4.—*Inoculations with three strains of Phytomonas beticola (Nos. 1, 2, and 3) and P. tumefaciens (M. S. C. No. 146)*

Host	Organism and strains	Method of inoculation	No. of plants	No. of plants showing overgrowths			
				5 days	10 days	15 days	20 days
Sugar beet	Check	Wounded	12	0	0	0	0
" "	*P. beticola* 1	Wounded	25	0	10	12	24
" "	Check	Wounded	10	0	0	0	0
" "	*P. beticola* 3	Wounded	25	0	5	14	25
" "	*P. beticola* 3	Nonwounded	25	0	0	0	0
" "	Check	Nonwounded	25	0	0	0	0
" "	*P. beticola* 2	Wounded	25	0	0	0	0
Garden "	*P. beticola* 2	Wounded	25	0	3	4	9
Sugar "	*P. tumefaciens*	Wounded	25	0	0	0	15
" "	*P. tumefaciens*	Nonwounded	25	0	0	0	0

the case of two strains of *P. beticola* practically 100 per cent infection resulted in sugar beets. Inoculations without wounding failed in all cases; no galls appeared spontaneously on the check beets, wounded or nonwounded. Strain 2 of *P. beticola* was not pathogenic to sugar beets but did attack garden beets (Fig. 1, E). Crown gall was produced on 15 out of the 25 injured plants, and this figure doubtless would have been greater had a longer period been given for the galls to develop. From this test the significance of wounding to disease production is evident.

OVERWINTERING OF THE ORGANISM IN THE GALLS AND THE SOIL

In common agricultural practice, beets are topped in the field to the end that the débris containing *Phytomonas beticola* is scattered in the field.

[3] Letter to G. H. Coons.

Such galls on the surface of the soil would be subjected to drying and freezing, but the organisms within the galls would be more or less protected. To follow in a general way the effect of drying and low temperature upon masses of the organism in the galls, three tubercles from a beet that had been kept in the ice box (about 10° C.) from October 16, 1926, to January 15, 1927, were ground in a meat grinder. This material was mixed thoroughly with steam-sterilized soil from the greenhouse, having a pH reaction of 6.80. Ten pots were used and the following quantities of the ground material were added to the various pots of the series: 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 30.0, 50.0, 100.0, and 200.0 grams. Young sugar beets showing about 7 sets of leaves and whose root bundles had started to differentiate into the collateral type were transplanted, three plants to each pot. After the plants were firmly established, the dirt was removed from one side of the root. The crown and root were injured by scratching along the root with a sterile needle, so as to expose both the crown and lower portions of the beet to the material to be tested. The pots were watered uniformly with sterile water over a 27-day period. Thereafter, the plants received ordinary care. The results are given in table 5.

TABLE 5.—*Infections secured in a 40-day period with varying amounts of ground beet galls (Phytomonas beticola). Young sugar beets exposed to infection after being wounded at crown and along the roots*

Pot	Quantity	40 days	Galls produced
1	0.5 gm.	0	0
2	1.0 "	0	0
3	2.0 gms.	0	0
4	5.0 "	+	2
5	10.0 "	+	2
6	20.0 "	+	3
7	30.0 "	+	3 (4)[a]
8	50.0 "	+	3
9	100.0 "	+	3
10	200.0 "	+	3 (4)[a]

[a] Doubtful, *Phytomonas beticola* not recovered.

The persistence of the organism in galled beets which were stored at relatively low temperature and with considerable drying is shown by this test. The failure to secure infection with the smaller quantities of the ground sugar-beet gall indicates a reduction in the number of germs under the conditions, but the reduction probably is not significant in the field relations of the organism.

In many fields, in Colorado, fall plowing is practiced. Under such conditions the diseased material would be covered with soil. To determine whether the organism survives in the crowns when covered with soil, two beets showing galls were placed out of doors on November 3, 1926, and were buried in sandy loam soil at a depth of 6 inches. After being subjected to ordinary winter conditions in Michigan, with alternate freezing and thawing (lowest temperature – 10° F.), one of these beets was examined March 3, 1927, and the other April 29, 1927, and both were found somewhat disintegrated. Isolations made from the interior of the decaying galls gave a great variety of organisms. From these a large number of yellow organisms were selected, some of which were shown by cultural tests and inoculations to be *Phytomonas beticola*. It is evident from this test that the bacteria in the galls buried in the soil can withstand severe winter conditions.

Aside from the persistence of *Phytomonas beticola* in the galls where more or less protection of the masses of bacteria is given by the beet tissue, it is evident that the organism may exist saprophytically in the soil as a free living form. In order to investigate this phase, the following preliminary test was carried on to test the technique of determination of suspected isolations by use of an agglutinating serum as proposed by Goldsworthy (4).

A composite broth mixture of *Phytomonas beticola* No. 1 and several other undetermined yellow organisms which had been isolated from soil at Rocky Ford, Colorado, was poured onto soil in flower pots in which young sugar-beet plants were growing. The beets were then punctured with a sterile needle and kept moist with sterile water; they later developed galls. Fourteen days after the suspension was poured on the soil, cultures were made from the soil in serial dilutions, as shown in table 6. The numerous yellow organisms which developed on the plates were isolated and grown on

TABLE 6.—*Test of a number of yellow organisms obtained from dilution plates of soil, against a serum agglutinating Phytomonas beticola at a titre of 1:1200*

Soil dilutions used	Yellow colonies selected	Number agglutinated by *P. beticola* serum
1/1,000	14	4
1/10,000	9	3
1/100,000	1	0
1/1,000,000	2	0
1/2,000,000	1	0
1/5,000,000	3	1

potato-dextrose agar and then tested with a *P. beticola* antiserum in the usual manner (Table 6).

It will be seen that a total of 8 organisms out of the 30 suspected isolations were agglutinated by the serum used.

These organisms were then used for inoculating rapidly growing sugar beets. One month after inoculation, seven of the eight organisms tested produced typical tubercles, thus confirming the preliminary diagnosis in seven cases out of eight. The organism failing to produce the disease in sugar beets may or may not have been *Phytomonas beticola*. The results of this test corroborate Goldsworthy's claim as to the value of agglutinating serum for quick diagnosis of suspects and indicate that suspected isolations from field soils could be readily diagnosed.

The method was then used in field tests. The first work was done with soil samples from a field at Lamar, Colorado, in which oats were growing and which, in the previous year, had shown considerable bacterial-pocket disease (10 per cent in certain portions). Twenty samples taken at random places in the field July 2, 1927, were used in the test. Serial dilutions of 1–10,000 to 1–2,000,000 were made in duplicate of each sample and these were employed in seeding potato-dextrose-agar (pH 7.56) plates. From these plates, 89 organisms showing the most typical pigment formation and growth characteristics were picked out and cultured. These 89 suspected organisms were then tested by use of the serum known to agglutinate *Phytomonas beticola* at a titre of 2200. Six showed a strong agglutination reaction with the test serum at a 1–1,000 dilution and were provisionally considered as being *P. beticola* (Table 7). The pathogenicity of each of the six organisms was then tested by wounding and inoculating rapidly growing sugar beets and garden beets in the experimental field. Many other beets were similarly wounded as a check on spontaneous occurrence of galls. No galls appeared on these latter beets. Five of the six organisms selected produced galls on sugar beets, and all of the organisms proved pathogenic to garden beets, thus confirming the preliminary diagnosis based upon the agglutination reaction. It is evident that *P. beticola* had persisted over winter in the soil under the dry conditions prevalent in the Colorado district, and the technique as outlined by Goldsworthy proved useful in field studies. Without using an excessive number of dilution plates, *P. beticola* was isolated from soil of a field where the bacterial-pocket disease had occurred on sugar beets the previous year.

Other soil samples taken from the various Rocky Ford, Colorado, fields where the bacterial-pocket disease was uncommon or where beets had not been grown at all recently yielded only one definitely identified culture of *Phytomonas beticola*, although many hundred platings were made. Irriga-

TABLE 7.—*Results of the agglutination test with Phytomonas beticola antiserum and organisms isolated from the twenty samples obtained at Lamar, Colorado. Record for colonies given as averages*

Soil dilutions used for plates	Plates		Serum dilutions				
	Number of colonies Average	Number of yellow colonies Average	Number of yellow colonies showing agglutination				
			1:5	1:50	1:100	1:500	1:1000
1/1,000	Too numerous	Too numerous					
1/1,000	" "	" "		None selected			
1/10,000	96	27	2	1	0	0	0
1/10,000	103	16	6	4	2	2	2[a]
1/50,000	47	22	2	1	1	1	1[b]
1/50,000	30	11	1	1	0	0	0
1/100,000	12	7	1	1	1	1	1[a]
1/100,000	17	3	1	?	?	?	1[a]
1/1,000,000	4	1	0	0	0	0	0
1/1,000,000	8	0	0	0	0	0	0
1/2,000,000	9	2	1	1	1	1	1[a]
1/2,000,000	5	0	0	0	0	0	0
Totals	331	89	14	9	5	5	6

[a] Produced galls on sugar beets and garden beets typical of *Phytomonas beticola*.

[b] Not pathogenic to suger beets, produced typical *Phytomonas beticola* galls in garden beet.

tion water from the Rocky Ford and Catlin systems[4] was tested by the above method, but in no cases were agglutinating organisms secured. Soil that as far as is known may be termed virgin soil gave no agglutinating organisms; however, it is probable that the amount of material tested was insufficient to prove that the organism is not present in that type of soil. No conclusion is warranted as to the natural occurrence of the organism in western soils, the one positive isolation being from a cultivated field on which beets may have been grown at some earlier time. It seems difficult to recover the organism except from fields with considerable infestation, and, if the organism is endemic in western soils, sugar-beet culture probably serves to intensify the infestation.

The above experiments, taken in conjunction with the overwintering results previously outlined, give evidence that *Phytomonas beticola* can overwinter in Colorado soils under ordinary climatic conditions. In the soil samples taken from the Colorado fields no recognizable fragments of

[4] Two irrigation systems supplying water to fields in the Arkansas Valley, Colorado. The water from the river is run onto fields at the upper end of the valley, and the waste water from these fields is used on the fields lower down the valley.

beets were included in the material plated, and the organisms are believed to have been free living forms. This evidence, coupled with the development of the disease here and there in the Arkansas Valley, leads the writer to the opinion that with this organism we have to do with a pathogene capable of living in the soil from season to season if not for longer periods.

EXPERIMENTAL WORK WITH STRAINS OF PHYTOMONAS BETICOLA

In the course of these studies a number of organisms have been isolated, tested for pathogenicity, and used in cultural and serological tests. These isolations have shown certain recognizable characteristics, and, for convenience, the organisms are discussed under the laboratory number. All conform closely to the cultural criteria for the species and have varied chiefly in pathogenicity, in rate of growth, and in intensity of pigment.

No. 1 was isolated from a sugar beet received from Rocky Ford, Colorado, October 16, 1926. It produced abundant growth in media and was strongly virulent in a series of inoculation tests.

No. 1-a was reisolated from a sugar beet inoculated with strain No. 1. The organism made good growth on laboratory media and showed strong virulence for sugar and garden beets as well as Swiss chard. "R" types were profuse.

No. 1-aa was reisolated from a sugar beet artificially inoculated with No. 1-a. Reactions were the same as for No. 1-a, but R and "S" type colonies were present in about equal numbers.

No. 2 was isolated by Mrs. M. C. Strong, of Michigan State College, from a beet sent from Lamar, Colorado, October 13, 1926. This organism produced good growth on laboratory media. It was distinct from many other strains in showing strong virulence on garden beets but was not virulent to sugar beets in repeated inoculation tests.

No. 2-a was reisolated during the summer of 1928 from a garden beet at Rocky Ford, Colorado, which had been inoculated with strain No. 2. This organism, like strain 2, was virulent to garden beets, but nonvirulent to sugar beets in repeated tests.

No. 3 was isolated from soil obtained at Lamar, Colorado, as described in a previous section. The organism produced good growth on laboratory media and exhibited strong virulence to both sugar and garden beets.

Phytomonas tumefaciens, M. S. C. No. 146. A culture obtained from Dr. E. F. Smith, of the Laboratory of Plant Pathology, U. S. Department of Agriculture, and labelled "Hop Strain," has been maintained in stock culture for some years at Michigan State College, and was used in comparison with *Phytomonas beticola*.

The recent work published by Brown (1) as to cultural work, pathogenicity, and host considerations make unnecessary extended record of

findings in cultural studies with this organism. The standard cultural tests were used and, in every important particular, similar and confirmatory results were obtained. The characterization of *Phytomonas beticola* as given by Miss Brown seems adequate to permit sure diagnosis and differentiation.

An abridged statement as to pathogenicity, which also confirms Miss Brown's findings, may be given: The cultivated sugar beet, *Beta vulgaris* L., as represented by two varieties grown commercially at Rocky Ford, Colorado, namely, Pioneer and Flat Foliage, were found very susceptible to the bacterial-pocket disease discussed in this paper. No varietal differences as to resistance or susceptibility were found in sixteen varieties of commercial beets which were tested by artificial inoculation. Typical overgrowths also have been produced on garden beets, *B. vulgaris*, and Swiss chard, *B. vulgaris cicla* L. When inoculations were made into tomatoes, *Lycopersicon esculentum* Mill., no typical gall formation resulted. However, a small, smooth, yellowish spot on the stem at the point of injection (Fig. 2, D, left) was noticed 28 days after inoculation. Pure cultures of the organism were obtained from this spot at that time. It is probable that the tomato merely harbored the organism at the place of inoculation without definite invasion occurring. Tobacco, raspberry, apple, and geranium plants were inoculated but showed no symptoms of gall formation. In contrast to these results, all the above-mentioned plants with the exception of tobacco developed overgrowths when inoculated with the crown-gall organism in a series of tests paralleling the above-mentioned inoculations (Fig. 2, D, right). No wild host for the organism has been found, although various weeds belonging to the family Chenopodiaceae or closely related families were examined in fields where the disease was developing in considerable amount on the sugar beet.

SEROLOGICAL TESTS WITH THE VARIOUS STRAINS OF PHYTOMONAS BETICOLA AND OTHER BACTERIAL PATHOGENES

Agglutination Tests. Several writers have reported use of specific agglutinating sera as a method of identification of phytopathogenic bacteria (6, 7, 8). The writer's studies have been concerned with the production of specific agglutinating sera for *Phytomonas beticola*, for use in diagnosis of suspected organisms and in comparative tests in which other plant pathogenes were included. The methods followed were, in general, similar to those given by Zinsser *et al.* (12) and Sharp (8), but it was found, however, that if dead or heat-inactivated *P. beticola* was used as antigenes the sera obtained were not of so high a titre as was later obtained by using the living organisms. The organisms used as antigenes consisted of various

strains of *P. beticola*, a culture of *P. tumefaciens* (Hop Strain from E. F. Smith), and a culture of *Erwinia carotovora* (heated). Forty-eight-hour-old cultures on potato-dextrose agar (pH 7.2) were washed from the slant with normal saline, and, after filtering through sterile cotton and shaken with beads, were adjusted to a standard density (No. 2) by use of a McFarland nephelometer.

In the first attempts the standardized suspensions of *Phytomonas beticola* were heated to 56° C. for one hour before the intravenous injections. The titres obtained with this material were not very high, namely, 320 and 640. In another series of rabbits the antigene was injected without the preliminary heating. After 5 intravenous injections of 1 cc. of the antigene at 2- to 4-day intervals, sera of much higher titre (2200 to 3600) were obtained, as shown in the table.

Having obtained the high-titre sera, various plant and soil bacteria were subjected to cross-agglutination tests in which the macroscopic method of determination was used. Difficulty was experienced with *Phytomonas tumefaciens* because of its tendency to form small clumps spontaneously in the 0.85 per cent salt solution (*cf.* Link and Link, 6). With a suspension in 0.5 per cent salt solution centrifugalized to remove the heavy clumps, a fairly stable suspension was obtained.

TABLE 8.—*Antisera from rabbits used in agglutination tests: 5 injections, 1 cc. each, of nonheated bacterial suspension, at 2- to 4-day intervals were given*

Serum No.	Antigene			Method	Titre obtained
407[a]	*Phytomonas beticola*		No. 1	Intravenous	1200
239	"	"	" 1-a		2200
101	"	*tumefaciens*	" 146	"	2750
397	"	"	" 146	"	3050
294	"	*beticola*	" 1-a		3250
295	"	"	" 2	"	3600
395	*Erwinia carotovora*			Intraperitoneal	640

[a] Sera were given a number corresponding to the animal number at Michigan State College.

In table 8 the results of the agglutination tests are given. In each case, the antiserum was used with its homologous antigene, and in all cases except with the *Erwinia carotovora* serum, which was known to be of low titre, strong flocculation in dilutions up to 1 to 640 was obtained. When other strains or species were used, the agglutination was either much weaker or

TABLE 9.—*Results of agglutination tests in which the various antisera and a number of bacterial antigenes were employed*

Antigene	Antiserum 239, homologous with *Phytomonas beticola* No. 1-a							Antiserum 397, homologous with *Phytomonas tumefaciens* No. 146							Antiserum 395, homologous with *Erwinia carotovora*						
	Dilutions							Dilutions							Dilutions						
	1/10	1/20	1/40	1/160	1/320	1/640	Check	1/10	1/20	1/40	1/160	1/320	1/640	Check	1/10	1/20	1/40	1/160	1/320	1/640	Check
P. beticola No. 1-a	++++	++++	++++	++++	++++	++++	−	+	+	−	−	−	−	−	±	−	−	−	−	−	−
P. beticola No. 2-a	+	+	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
P. beticola No. 2	+	+	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−
B. noctuarum[a]	−	−	−	−	−	−	−	−	−	−	−	−	−	−							
E. atroseptica (1)	−	−	−	−	−	−	−	−	−	−	−	−	−	−							
E. atroseptica (2)	−	−	−	−	−	−	−	−	−	−	−	−	−	−							
P. vignae	+	±	−	−	−	−	−	+++	+	++	±	−	−	−	−	−	−	−	−	−	−
B. sphingidis[a]	−	−	−	−	−	−	−	−	−	−	−	−	−	−							
E. carotovora	±	−	−	−	−	−	−	−	−	−	−	−	−	−	++++	++++	++++	++++	++++	++	−
P. tumefaciens	±	±	−	−	−	−	−	++++	++++	++++	++++	+++	++++	−	+++	++	++	±	−	−	−

++++ Strong flocculations.
+++ Medium strong flocculations.
++ Medium flocculation.
+ Slight flocculation.
± Doubtful flocculation.
− No flocculation.
[a] Pathogenic to insects, obtained from G. F. White.

absent, the differences being so pronounced as to indicate clearly specificity of reaction. The following reactions deserve comment.

The antisera, 239 and 294, obtained by using *Phytomonas beticola* No. 1-a as an antigene, showed no strong reaction with any antigene except the homologous one (Tables 9 and 10). However, strains of *P. beticola* No. 2 and 2-a, isolated from typical bacterial-pocket galls on beets grown at Lamar and Rocky Ford, Colorado, respectively, showed slight flocculation in a dilution of 1–20. *Erwinia carotovora* showed a very slight tendency toward a reaction, but agglutination could be detected only with micro-agglutination methods. *Phytomonas vignae* showed a slight reaction with serum from rabbit 239, but from 294 no reaction was noticed. This organism, much the same as *P. tumefaciens*, has a tendency at times to flocculate in 0.85 salt solution, which may account for the doubtful reactions noted.

The antiserum of *Phytomonas tumefaciens* (Table 9) proved to be specific for its own antigene, except in the case of a 1–40 dilution reaction with *P. vignae* and 1–20 with *P. beticola* No. 1-a. The reaction was very marked in the case of *P. vignae*, and a group relationship between these two organisms may exist. The agglutination with *P. beticola* No. 1-a was very slight, and no strong group agglutination was indicated.

The *Erwinia carotovora* antiserum 395, which was the result of intraperitoneal injections, gave a very low titre. However, it was specific in its action. Except when *Phytomonas tumefaciens* was used as an antigene, there was a definite reaction up to 1–40 dilution. Thus, an apparently contradictory situation was observed, since the antiserum homologous with *P. tumefaciens* in the previous experiment had shown no agglutinative power for *E. carotovora*. Link and Link (6) have previously observed the same phenomenon. It was later found that partial agglutinins for *P. tumefaciens* were actually present in the serum homologous for *E. carotovora*. This was determined by making separate additions of *E. carotovora* and *P. tumefaciens* emulsions, which are concerned in the group agglutination, to aliquots of antiserum. The homologous antigene (*E. carotovora*) when added to the antiserum 395 and incubated for two hours then centrifugalized to obtain a clear serum. This serum was then tested again with the *E. carotovora* and *P. tumefaciens*. No agglutination was obtained with either organism. When the attempt was made to secure absorption, using a suspension of *P. tumefaciens* with another part of the serum in the same dilution, the specific agglutinins for *E. carotovora* were not removed and it was found that the clear serum obtained after incubation and centrifugalization was still capable of agglutinating *E. carotovora*. The clear serum did not agglutinate *P. tumefaciens*. This test is interpreted to indicate that a specific protein is present in *E. carotovora* for the production of

TABLE 10.—*Test of Phytomonas beticola No. 2 and Phytomonas beticola No. 1-a antiserum with various antigenes*

Antigene	Antiserum 295, homologous with *Phytomonas beticola* No. 2							Antiserum 294, homologous with *Phytomonas beticola* No. 1-a						
	Dilutions							Dilutions						
	1/10	1/20	1/40	1/160	1/320	1/640	Check	1/10	1/20	1/40	1/160	1/320	1/640	Check
P. beticola No. 1-a	−	−	−	−	−	−	−	++++	++++	++++	++++	++++	++++	−
P. beticola No. 2-a	++++	++++	++++	++++	++++	++++	−							
P. tumfaciens No. 146	−	−	−	−	−	−	−	±	±	−	−	−	−	−
P. beticola No. 2	++++	++++	++++	++++	++++	++++	−	++	+	+	−	−	−	−
P. beticola No. 1-aa								++++	++++	++++	++++	++++	++++	−
E. atroseptica (1)								−	−	−	−	−	−	−
E. atroseptica (2)								−	−	−	−	−	−	−
E. amylovora								−	−	−	−	−	−	−
Ps. fluorescens								−	−	−	−	−	−	−
E. carotovora								−	−	−	−	−	−	−
P. vignae								−	−	−	−	−	−	−
P. hyacinthi								−	−	−	−	−	−	−

++++ Strong flocculations.
++ Medium flocculation.
+ Slight flocculation.
± Doubtful flocculation.
− No flocculation.

antibodies that will react weakly with *P. tumefaciens*, but the reaction is not reciprocal, *i.e.*, *P. tumefaciens* does not contain antigenic material capable of producing a serum reactive with *E. carotovora*.

The *P. beticola* No. 2 antiserum agglutinated only the homologous antigene and the subculture of serum No. 2, 2-a. No agglutination whatsoever was obtained in cross-agglutination tests in which *P. beticola* No. 1-a or *P. tumefaciens* was employed as an antigene (Table 10).

The antiserum of *P. beticola* No. 1-a from rabbit 294 showed a slight tendency toward agglutination with 1–10 dilution of *P. tumefaciens* and a slight reaction up to 1–40 with strain No. 2. *Phytomonas beticola* 1-aa, which was a reisolation of No. 1-a, when used as an antigene, gave the same agglutination as the parent strain.

The work has shown that sera of relatively high titre are extremely useful for diagnostic purposes if decision is based upon positive agglutinations obtained in high dilutions. *Phytomonas beticola* antisera when tested against other plant pathogenes as antigenes proved to be specific. The negative results of cross-agglutination tests when *P. beticola* strain 2 and 1-a were reciprocally used with the appropriate heterologous antiserum are not unlike results of agglutination tests recorded for strains known to exist within other bacterial species. Strongly positive reactions in high dilutions are apparently significant in showing serological and presumptive specific identity. The negative or weakly positive reactions indicate difference in the antigenes but give little clue to the extent of these differences.

CULTURAL CHARACTERISTICS AND SEROLOGICAL REACTIONS OF R AND S TYPE COLONIES OF PHYTOMONAS BETICOLA

In cultural work with *Phytomonas beticola* No. 1-a there appeared on many plates two distinct types of colonies and in a few rare cases a third type. One was round, regular, yellow, and characterized by a smooth glistening surface; the other more or less irregular, of cheesy consistency, and with a rough or wrinkled surface. Following the terminology of recent bacteriological investigations (*cf.* Hadley 5), the former was termed the S type (smooth), the latter the R type (rough). The third was different from either of the above-mentioned forms and had a rhizoid or lobed outline; and was therefore called the rhizoid type. These three forms have been described by Brown (1) in her recent work and all three forms found to be infectious. In hanging-drop preparations the bacteria from the S types were very motile. The bacteria from the R-type colonies were distinctly more sluggish.

Cultures of either R or S type growing on agar slants remained rather constant to their growth type when regular 24-hour transfers were made.

Dilution plates made from the older (10–20 days) R-type cultures also showed considerable constancy in the types present, since only occasionally the S-type colony appeared on the plates (Table 11).

TABLE 11.—*Types of colonies appearing on dilution plates made from 20-day-old R- and S-type cultures. Potato-dextrose agar used in plates 150 x 26 mm.*

a	Parent culture of R type				b	Parent culture of S type			
Plate	R type	S type	Sub-merged	Per cent S type	Plate	R type	S type	Sub-merged	Per cent R type
1-a	6	0	21	0	1-b	10	15	15	40.0
2-a	18	1	12	5.2	2-b	11	9	15	55.0
3-a	10	0	20	0	3-b	10	16	16	38.4
4-a	20	0	9	0	4-b	11	16	8	40.7
5-a	17	0	11	0	5-b	19	19	8	50.0
6-a	19	0	8	0	6-b	6	11	11	35.2
7-a	23	1	8	4.1	7-b	6	16	9	27.2
8-a	42	0	16	0	8-b	23	21	20	52.2
9-a	33	0	15	0	9-b	24	29	12	45.2
10-a	19	2	19	9.5	10-b	7	19	21	26.9
				1.8					41.0

On the other hand, the S-type cultures which had shown constancy-of-growth forms in the 24-hour transfers and which appeared as typical smooth growths, in older tubes gave in a preliminary test, when dilution plates were made from these old tubes, a very large number (approximately 41 per cent) of R-type colonies among the S-type colonies (Table 12).

TABLE 12.—*Number of R- and S-type colonies resulting from selection of the sixth subculture of S type of Phytomonas beticola on dilution plates (Transfers made every 24 hours)*

Plate number	Colonies			Remarks
	O–R	S	Submerged	
1	0	2	12	
2	0	13	13	
3	0	10	8	
4	2	19	6	1 R- and 1 O-type[a]
5	0	13	1	
6	1	2	7	O-type[a]
7	0	6	7	
8	0	9	3	
9	0	10	11	
10	0	23	5	

[a] Lobe-type colonies distinct from R- or S-type.

Further work was done to substantiate the above findings; 60 dilution plates were made from many other 20-day-old S-type cultures. The results from these checked well with the first test, since 42.7 ± 4.24 per cent R-type colonies were found to be present among the S-type colonies. It would seem that under conditions present in old culture tubes, the S-type growth form is replaced strongly by the R type and that the R-type growth form is rather permanent.

Certain S-type colonies were selected from the dilution plates and were studied after six transplants to agar slants at 24-hour intervals. All of these cultures appeared to be of the S type in the test-tube cultures. In table 12 the result of a plating from one of these tubes is given. It will be seen that the colonies are prevailingly of the S type, but one R and two rhizoid types appeared.

Very few colonies of rhizoid type have appeared in numerous platings of this organism, the chief colony types being the R and S growths. These are generally clear-cut in their essential features and readily distinguishable. In a few cases there was observed distinct intergradations and these took on some definite aspects. In a number of colonies on the dilution plates made from old cultures of S slants, showing both R and S types, side outcropping of R type was often noticed on S-type colonies. In all instances these outcrops were located near the edge of the smooth colony in a three- or four-day-old plate. A smooth portion was never observed surrounding an entire R-type colony. It was impossible to tell if the two types started to grow in conjunction with each other or whether it was a natural outcrop. Dilutions made from these outcrops always gave a great number of both R and S types.

Attempt was made to differentiate serologically between R and S types of *Phytomonas beticola*. The agglutination and precipitin tests were employed.

The method followed in obtaining a high-titre serum was the same as in the previous discussion on agglutination test. The antigene was obtained by picking out either R or S type from dilution plates. The R antigene when suspended in saline solution had a tendency to clump and settle out, even after three washings. It, however, was used to inject rabbits. The S type remained in suspension without clumping.

The resultant sera from animals injected with the S type showed an average titre of 3600. Sera homologous with the R-type antigene when tested with S antigene gave 1200 titre. It was found that an agglutination test with an R-type antigene was impossible, because of the spontaneous agglutination in various dilutions of salt solution and distilled water. For this reason, if serological differences exist between R and S type they could

not be detected by the agglutination test. It may be pointed out that, judging from the titre of sera obtained with the S type and the R type used as an antigene, the degree of antigenic activity of the S type was much greater than that of the R type. In repeated tests in which the isolations of the S-type and R-type organisms were from the same parent culture, the antisera from the S-injected animals were always much higher in titre than those of the R organisms; the agglutination test being made with the original parent culture as the antigene. A precipitin test was utilized to evaluate the sera homologous with the R type, following the method outlined by Sharp (8). The antigenes were obtained by growing the R and S forms in nutrient broth for 10 days, and the ordinary precipitin procedure was carried out with the clear filtrate (Table 13).

TABLE 13.—*Precipitin test in which broth filtrates of R and S type of Phytomonas beticola were employed against an R-type antiserum*

Type of filtrate	Amounts		Results
	Filtrate	Antiserum	
S	1.0 cc.	1.0 cc.	++++
S	.5 cc.	.5 cc.	++++
S	.25 cc.	.125 cc.	++++
S	.125 cc.	.05 cc.	+
Control[a]	1.0 cc.	1.0 cc.	–
Control[a]	.5 cc.	.5 cc.	–
R	1.0 cc.	1.0 cc.	++++
R	.5 cc.	.5 cc.	++++
R	.25 cc.	.125 cc.	++
R	.125 cc.	.05 cc.	+

[a] Sterile broth filtrate.

The test presented in table 13 shows that the bacterial proteins or proteins that cause the production of the precipitins are so closely related in R and S types that any serological differences could not be differentiated by this test. Similar results were obtained from 6-, 7-, and 8-day-old cultures used as antigenes.

The Pathogenicity and Virulence of R and S Types. The R- and S-type organisms used in plant inoculations were suspensions from 24-hour agar slants, which had been picked from dilution plates of the virulent strain No. 1-aa in the previously described test for constancy of type and which had since been transferred daily.

The sugar-beet plants inoculated were selected from a lot of the commercial variety Pioneer on the U. S. Department of Agriculture Field Lab-

oratory plots at Rocky Ford, Colorado. All beets were in good growing condition and were approximately of the same size. The environmental conditions were uniform and favorable for disease development.

The sugar-beet roots were inoculated near the crown with a hypodermic syringe. A clean sterile syringe and needle were used for each type of inoculum. Irrigation water was kept from the beets for three days, then thorough irrigation was given. The withholding of water was a precaution against washing *Phytomonas beticola* occurring naturally in the soil into the uncalloused wounds of the roots. Irrigation was necessary later to insure the rapid plant growth, so essential to the development of the disease. Readings were made 60 days after inoculations. The actual number of infected beets (Table 14) was practically the same with both types. The

TABLE 14.—*Summary of data on the pathogenicity of R and S types of Phytomonas beticola on commercial sugar beets*

Row No.	Inoculum	Sugar beets			Remarks
		Number used	Galled	Not galled	
1	S	92	60	30	Small galls
2	Check	104	5	95	[a]
3	R	96	65	31	Large galls
4	No. 1-aa (stock culture)	98	8	89	Old cultures
5	" 1-aa " "	25	24	0	24-hr. cultures
6	Check	129	3	118	[b]

[a] Three of these (5) were plow-injured.

[b] Two of these (3) were plow-injured.

results of this experiment showed that the forms are equally pathogenic. If the size of gall is taken as a criterion, then the R forms differ strongly in effect on the host from the S forms. The difference in size of the overgrowths was very marked and, without exception, roots inoculated with the R type showed much larger galls than those inoculated with the S type.

Isolations from the galls arising from inoculations with either the R or S type gave both types on dilution plates, and subsequent inoculations of sugar beets with fresh isolation of the two types from plates made from galls of known origin showed gall formation irrespective of type or origin. No relation of colony type to pathogenicity was found. The findings of other workers on bacterial variations have indicated that the S types are

more virulent, and cases are recorded where the R type is nonpathogenic or attenuated. Sharp (8), working with *Bacterium phascoli sojense*, has found S types to be the more virulent to soybeans, judgment being based on measurement of spot size. The majority of workers using animal pathogenes have reported the S type as the more virulent. In a few cases exceptions to this situation have been recorded for animal pathogenes. One hypothesis to account for bacterial variation assigns the observed phenomenon to association of characters. For example, Dible (2) has suggested that sorting out of characters takes place in bacteria in different phases of variation, and this results in certain associations such as smoothness and virulence. Exceptions may occur and he recognizes that a character which normally goes with one type of colony may become divorced and be associated with another. Following this suggestion, it would seem at first glance that *Phytomonas beticola* presents one of the exceptional cases. The sugar beet appears to be very susceptible to *P. beticola* and, due to the low resistance, it is possible that it is not a good host plant for determining differences in pathogenicity. The inoculation experiments herein reported show that once infection takes place and the organism becomes established the R type produces the larger galls. If we accept gall size as an index of virulence, the R type could be considered the more virulent. The nature of overgrowth formation in the bacterial-pocket disease probably is one thing and the necrosis in the bacterial-spot disease another. Attempts to produce galls with filtrates have so far given negative results, and there is no evidence to indicate relationship of size of overgrowth to virulence. The fact that the R- and S-type cultures used in inoculation are not exclusively one type, as evidenced by platings both from the culture tubes and galls, makes it impossible to reach a definite conclusion.

The situation as to bacterial variations in *Phytomonas beticola*, so far as is known, is as follows:

Smooth Type (S)	*Rough Type (R)*
1. Colonies on agar plates smooth, glistening, and smooth in outline.	1. Colonies on agar plates rough, cheesy consistency, and often irregular in outline.
2. Suspension stable in physiological salt solution.	2. Agglutination takes place in physiological salt solution.
3. Motility, very marked.	3. Motility, very much reduced.
4. Growth uniformly turbid in broth with slight settling out.	4. Growth forms a heavy sediment in broth.
5. Pathogenic to beets. Overgrowths small.	5. Pathogenic to beets. Overgrowths larger than those from S type.
6. Antigenic properties very strong.	6. Antigenic properties not so strong as in S type.

Previous investigations (Link and Link (6), Sharp (8), Brown (1), and others) have shown the existence of variation in bacterial plant pathogenes which parallels the situation reported for other bacterial species, chiefly animal pathogenes. In the work here reported, the type of colony, whether R or S, was a striking expression of the existence of variation in *Phytomonas beticola*. Morphological and physiological differences were found associated with the difference in colony type. Of these differences, the almost complete absence of motility in the R type may be mentioned. Differences in pathogenicity between the R and S types exist, but conclusion as to the direction of the change cannot be made, since it is not known that the size of overgrowth produced is a measure of virulence. The antigenic properties of the R type are less than those of the S type, but, with present technique, no serological independence can be determined.

It is evident that variation in colony type and in reactions of an organism may occur during the course of a series of tests, and in experimental work this possibility must be recognized.

SUMMARY

1. The bacterial-pocket disease of sugar beets caused by *Phytomonas beticola* has been found in several additional western locations and in a few fields a considerable percentage of the plants were affected.

2. Comparative analyses of diseased beets and of normal beets show that the disease reduces the sugar percentage and the purity greatly.

3. The bacterial-pocket disease has commonly not been distinguished in field work from crown gall and a comparison of the two diseases is given as an aid to ready field diagnosis.

4. The organism enters the host through wounds, injuries from cultivating implements and hail being common entrance points.

5. The organism can overwinter in the overgrowths and as a free-living organism in the soil. In isolation of the organism from the soil, identification of suspects was made by the agglutination test and confirmed by inoculation tests with sugar and garden beets.

6. Serological tests with a number of bacterial species have shown the agglutination reaction to be specific and sera of high titre have been useful in diagnosing suspects, if decision is based upon strongly positive agglutination reactions obtained in high dilutions. Two strains of *Phytomonas beticola* were serologically independent in their agglutination reactions; therefore, negative results of agglutination tests are not to be interpreted as evidence of specific difference.

7. A strain of *Phytomonas beticola* was found to exhibit dissociation which manifested itself by variability in colony type, corresponding to the

rough and smooth types described for other species of bacteria. Subcultures of each type remained rather constant when 24-hour transfers were made, but 20-day-old cultures, originally of the S type, showed approximately 41 per cent R-type colonies in repeated tests. Where the parent culture was of the S type, a very low percentage (2 per cent average) of the colonies from 20-day-old cultures were of the R type.

8. The R-type cultures apparently had fewer antigenic properties than the S-type cultures. Reciprocal agglutination tests could not be made because of the spontaneous clumping of the R-type organisms in salt solution, but the R-type antiserum agglutinated the S-type culture. The R- and S-type antigenes, when used in a precipitin test with an R-type antiserum, gave identical results. Both forms are pathogenic to sugar beets, but the R-type cultures produced the largest overgrowths. It is concluded that *Phytomonas beticola* shows variation comparable to that recorded for other bacteria and this should be taken into account in experimental work.

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TARGET BLOTCH OF SUGAR CANE[1]

C. N. PRIODE[2]

During the past 38 years since Van Breda de Haan (1) described and illustrated what is now known as the eye-spot disease of sugar cane, considerable attention has been given to the leaf spots caused by different species of the Helminthosporium fungi. Until recent years some confusion has surrounded these maladies, because they generally have been considered to be different stages of the same disease.

Van Breda de Haan believed the eye-spot organism to be a species of Cercospora, and named it *C. sacchari.* The descriptions and colored plates published by Lucassen and Went (6) in 1894, by Walker and Went (8)[3] in 1898, and by Krugger (5) in 1899, however, show it to be a species of Helminthosporium and not a Cercospora.

In 1913 Butler and Hafiz (2)[4] reported a leaf spot of sugar cane from India which they called "Helminthosporiose." The fungus causing this disease they named *Helminthosporium sacchari* and it resembled somewhat, in general appearance, the *Cercospora sacchari* described by Van Breda de Haan as the organism causing the eye spot of sugar cane. The color of the spores, the spore measurements, and the descriptions and colored plates of the diseased specimens given by these two authors, however, show it to be a disease separate and distinct from the *C. sacchari* of Van Breda de Haan.

In 1927 Faris (4) described a new leaf spot of sugar cane in Cuba which he called "brown stripe," the name being suggested by the brown, straw-colored spots on the diseased leaves. This disease also is caused by a species of Helminthosporium, and, until the publication of Faris' experiments, was believed to be identical with the sugar-cane eye spot.

During the winter of 1927 what appeared to be an undescribed disease of sugar cane was observed on various plantations in Cuba. Isolation and inoculation experiments proved it to be a new disease caused by Helminthosporium, separate and distinct from any of those mentioned above. It had none of the characteristic symptoms of the eye spot, brown stripe, or Helminthosporiose, and did not readily attack some of the varieties known to be susceptible to these maladies. The incipient stages of the known diseases caused by Helminthosporium on sugar cane are very

[1] Scientific Contributions No. 18, Tropical Plant Research Foundation. From the Cuba Sugar Club Experiment Station, Baraguá, Province of Camagüey, Cuba.

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[3] See Pl. XXI, Figs. 1–5.

[4] See Pl. I, Fig. 3, and Pl. VI, Figs. 1–8.

much alike in that the infections appear as tiny red specks. This similarity of the early stages of infection is largely responsible for the confusion which has surrounded the identity of the eye spot, brown stripe, and Helminthosporiose. The new disease has this same similarity in its early stages, *i.e.*, infection appears as tiny, red specks where the fungus enters the leaf tissues. As the infection advances, however, characteristic symptoms are produced which readily distinguish it from the other diseases caused by Helminthosporium. Infection usually takes place on the leaf roll, resulting in large, necrotic blotches having irregular, concentric rings which roughly resemble a target board in appearance. Because of this resemblance the disease, in an earlier report by the writer (7), was named "target blotch."

The purpose of this article is to give a more detailed description of the symptoms and to report the results of some further experiments with the disease.

SYMPTOMS OF THE TARGET BLOTCH OF SUGAR CANE

The characteristic symptoms of target blotch are the zonate markings or irregular, concentric rings previously mentioned. These rings are always found on the rolled leaves in the leaf spindle, but the target-board effect is more pronounced after the leaf becomes detached from the leaf roll (Plate I, A). The number of rings produced on an infected leaf varies according to the severity of the infection and the susceptibility of the variety. In some cases only a few rings are formed before the leaf becomes separated from the leaf roll, while in other cases there may be ten or more rings around one infection center. Usually these rings continue to form as long as the leaves remain in a tightly rolled condition in the leaf spindle but cease to develop when the leaf becomes separated from the leaf roll. Infection usually starts with the germination of a spore on the leaf roll. The germ tube enters the leaf tissues, and in a very short time a tiny water-soaked speck may be seen around the point of entrance. As the infection advances the water-soaked area first becomes red, then changes to brown as the spot enlarges. Usually there is very little indication of a halo around the infected areas, but the brown spots soon become surrounded by a mottled, red ring (Plate I, B). As the fungus advances into the healthy tissues this mottled ring becomes necrotic and turns brown, forming the first of a series of brown, straw-colored, concentric rings. As each successive ring is formed by the death of the infected tissues another mottled, red ring is produced by the further advance of the fungus into the surrounding healthy cells. This process of ring development continues until the leaves unfold and separate from the leaf roll. The infection does not appear to spread to any appreciable extent on the unrolled leaves.

The infected area sometimes covers the entire width of the leaf and the target-board appearance is very noticeable (Plate I, A, and Fig. 1).

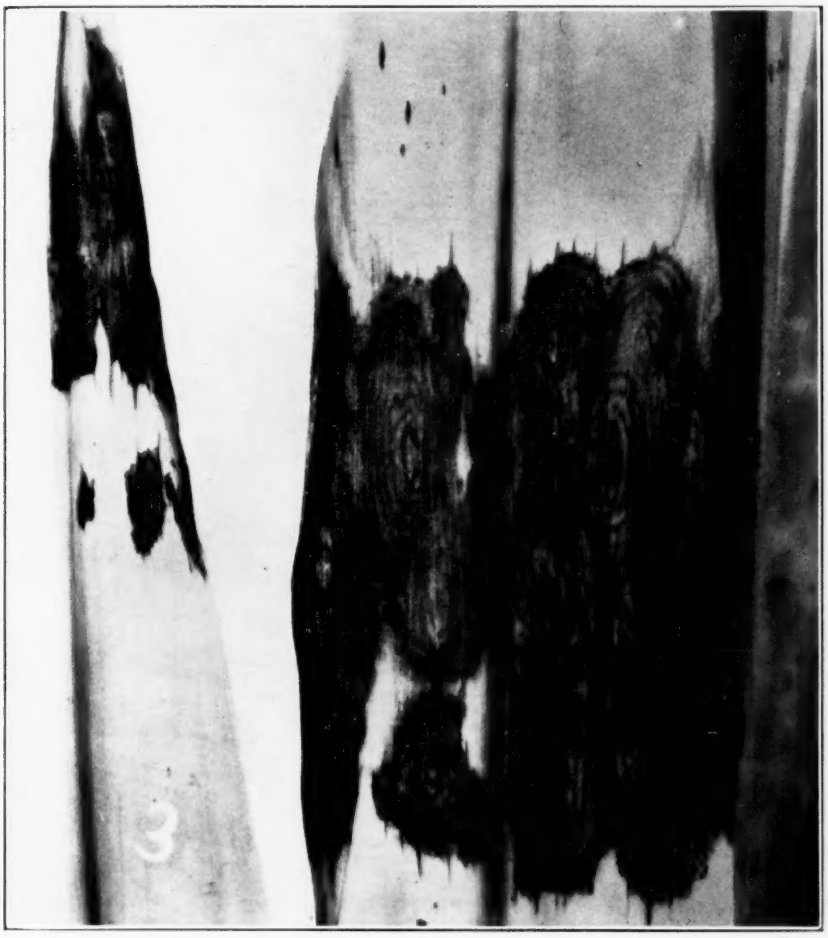

FIG. 1. Infected plant showing typical target blotch on the open leaf. Notice the blotch on the young leaf roll to the left.

The infection on the leaf roll is usually accompanied by a marked depression over the diseased spot caused by the shrinking of the tissues as the diseased cells die (Plate I, B). Infection on the leaf roll usually penetrates the successive layers, forming rings on all the young leaves in the roll. Sometimes the fungus penetrates two or more layers of the same rolled leaf and rings form at each point of penetration. When such a leaf is fully separated from the leaf spindle it appears to have two or more original in-

fection centers, while in reality they all started from the same point of infection. Each spot becomes smaller toward the center of the leaf roll. This is shown very well in Plate I, D. Cases are sometimes seen where several infection centers occur at different heights on the same leaf roll, as shown in plate I, B, and figure 2, A.

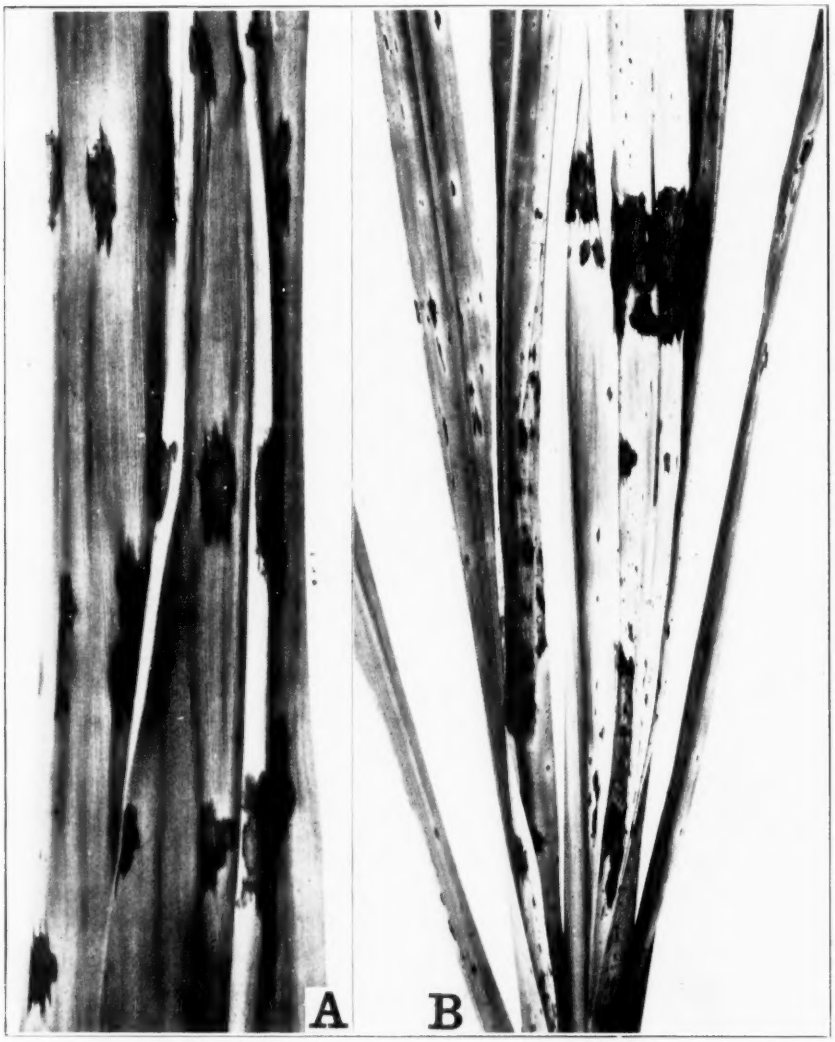

FIG. 2. A. Target-blotch infection occurring at different heights on the same leaf roll. B. Showing numerous small spots or infection centers on the young leaves, most of which resulted from the germination of the spores which were produced on the old blotches above.

On the more susceptible varieties the fungus sometimes penetrates to the center of the leaf roll and extends down to the growing point, causing the death of the whole plant top. Where such cases occur the leaves do not usually unroll but appear to be held together by a web-like mass of fungus mycelium. Spores are produced in large quantities on the old blotches. Numerous tiny red specks or spots can usually be seen on the young leaves below the old blotches. These result, apparently, from the germination of the spores that fall down from the old infections above (Plate I, C, and Fig. 2, B). Sometimes several of these small spots grow together, forming a larger spot or blotch of irregular size and shape. No leaf-sheath or stalk infections have been observed but the fungus has been observed to attack the leaf midrib very readily (Plate I, A). In this respect target blotch differs from both eye spot and brown stripe, neither of which disease attacks the midrib very readily.

ISOLATION OF THE ORGANISM

The first attempts to isolate an organism from plants affected with target blotch were made late in the winter of 1927. The usual method for making isolations was used. Pieces of diseased tissue were surface sterilized, planted in agar plates and incubated at room temperature. In nearly every instance a species of Helminthosporium was obtained which grew very rapidly in agar cultures. Plates planted with diseased pieces in the afternoons usually showed marked growth of fungus mycelium on the following mornings and typical Helminthosporium spores were produced within a short time. Inoculations from these spores on the leaves of vigorously growing plants (no other plants were available at the time) produced only tiny red specks very similar in appearance to the incipient stages of brown stripe and eye spot, but from these infections on older tissues no target blotches developed.

Previous observations having shown that the disease was most severe during the dry cool season and on the more mature canes, further work was suspended until the following December when the disease again made its appearance. At this time isolations were made by the same method as described above and again a Helminthosporium fungus was obtained in nearly every plate. Inoculations made by spraying the spores from some of these plates on the leaves of healthy plants produced typical target blotches, as were observed in the field. Pure cultures were obtained by single-spore isolations and inoculations from these also produced the typical symptoms of the disease. Numerous isolations were made from these infected plants and what appeared to be the same Helminthosporium was obtained in nearly every instance. The fungus is comparatively easy to isolate and grows and fruits very readily in plate cultures.

Pure cultures can nearly always be obtained by unrolling the outer layers of a section of the diseased leaf roll and using the innermost tissues for plating material. Contaminating organisms are seldom encountered when this method is used.

DESCRIPTION OF THE ORGANISM

The general appearance of the target-blotch fungus is not unlike that of the other Helminthosporium species. The color and shape of the conidia

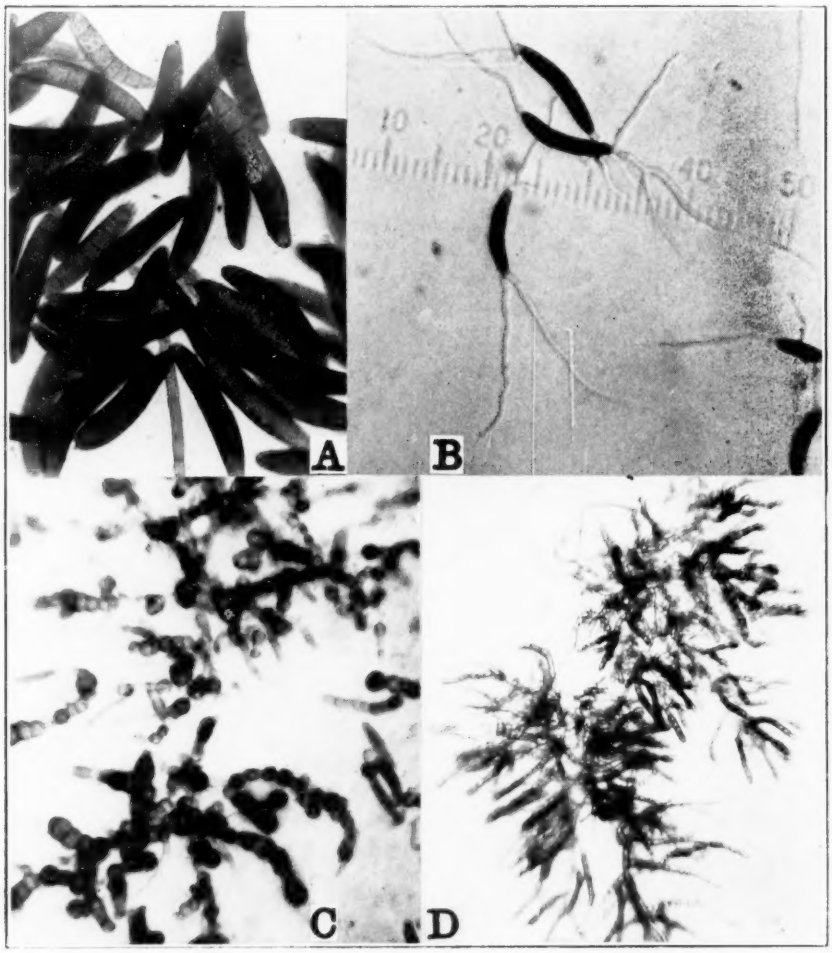

FIG. 3. A. Target-blotch spores from a 5% cane-juice agar culture. ×300. B. Germinating conidia of target blotch. ×112. C. Peculiar bead-like proliferations on the hyphal strands of the target-blotch fungus. Note the size of these growths as compared with the size of the spores. D. Finger-like projections found on the outer edges of certain target-blotch cultures.

on diseased material and on other media are typical of the Helminthosporium group and make easy the determination of the genus (Fig. 3, A). There are certain characteristics of this species, however, which distinguish it from the other species of the group.

The color of the mycelium usually is a whitish gray, but in some cases it may grade into brown or black. The strands which go down into the leaf tissues and the submerged strands in the plate cultures usually are a distinct brown. In petri-dish cultures the fungus makes an irregular, circular type of growth, spreading in all directions from the center. Concentric rings are produced on most media, although they may be almost indiscernible in certain cultures. These rings vary in width and number, according to the media used. On Czapek's agar the growth is very rapid and the rings are wide and few in number, while on 10 per cent cane-juice agar the rate of mycelial growth is rather slow and the rings produced are narrow and more numerous.

The mycelium has a distinctive branching habit, different branches often being connected by short mycelial strands. No swellings or other indications of a zygospore formation have been observed at these unions. Both the aerial and submerged portions of mycelium are numerously septate with varying distances between the septae.

There is also a wide variation in the relative widths of the individual strands, as some are very fine, while others appear coarse and fleshy. As a general rule the submerged portions are larger and more uniform in width than the aerial strands. This, probably, is due to the tendency of the aerial portions to dry out and collapse.

On the outer edge of certain cultures the submerged mycelia sometimes show numerous gnarls or swellings. The ends of the hyphal strands are divided into a great number of short sections by numerous septae. These swellings sometimes present peculiar, though interesting, formations in that the adjoining segments are of unequal shapes and sizes, some being considerably larger than the adjoining ones of the same strand. In some cases these formations consist of short strings of round, unequal-size, bead-like proliferations (Fig. 3, C). In other cases they are made up of finger-like projections of more uniform sizes and shapes (Fig. 3, D). From both of these peculiar types of mycelial proliferations, hyphal strands grow out in much the same way as germ tubes grow out from germinating conidiospores. These strands may arise from the terminal portions, from any intermediate segment, or from both, as is often the case. Within a short time spores typical of Helminthosporium are produced in great quantities on these new hyphal developments.

This peculiar mycelial growth has not been described as a growth characteristic of either the eye-spot or brown-stripe organism, nor is it com-

parable with the mycelial development of *H. giganteum*, described and illustrated by Drechsler (3). So far as the writer can determine, these peculiar growth characteristics have not been described for any other species of Helminthosporium.

There are considerable variations in the responses of the target-blotch fungus to the changes of medium and light. The growth characteristics on the different agars used in these experiments are as follows:

Growth in Czapek's Agar (*Fig. 4, A*). The mycelial growth on this medium is rather profuse and spreads rapidly over the agar surface. The aerial growth is grayish white to brown, while the surface mycelium is dark brown to black. Concentric rings are very pronounced, as shown in figure 4, A. The conidia on this medium are dark brown and usually of a granular appearance. They are rather sparsely produced on the aerial mycelia but very numerous on the surface growth.

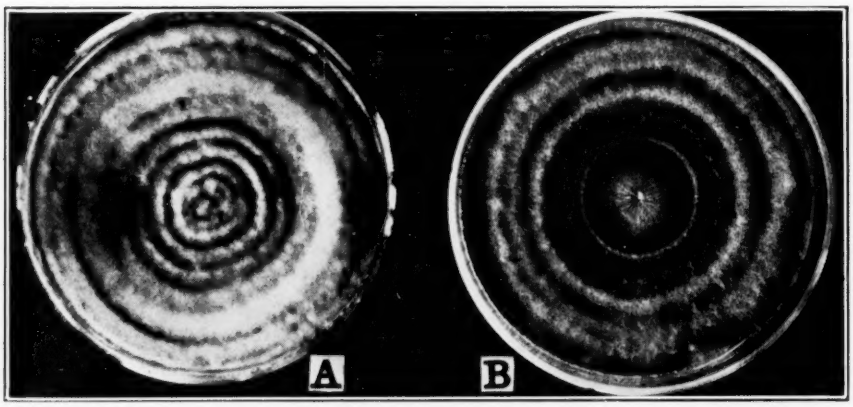

FIG. 4. A. Target-blotch culture on Czapek's agar showing distinct ring formation and considerable aerial growth. B. Culture on cornmeal agar, same age as culture A. The concentric rings are distinct but the aerial growth is not so profuse as on the Czapek's agar.

Growth in Corn-Meal Agar (*Fig. 4, B*). On this medium the mycelial growth is rather sparse but spreads rapidly from the center, forming distinct concentric rings. There usually is very little aerial growth, the mycelium lying close to the agar surface. The color is a light gray to faint greenish brown. The conidia are light brown, produced rather sparsely in center of culture but more abundantly on outer portions, usually forming a black ring around the outer edges of the culture.

Growth in 20% Cane-Juice Agar (*Fig. 5, A*). On this agar the mycelial growth is somewhat thin in center of culture but more profuse on

outer portions. Aerial growth is usually absent in the center but more prominent on the outer two-thirds of the culture. Concentric rings are usually produced, but in most cases are rather indistinct. Conidia are produced scatteringly over whole of culture but are found most abundantly on the surface growth around the outer edges. Both the mycelia and conidia are light brown.

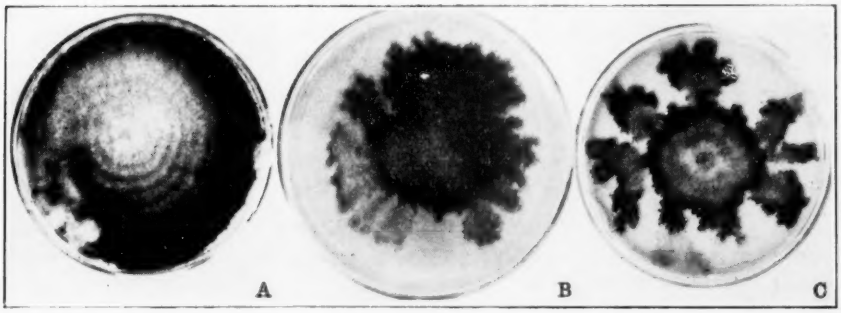

FIG. 5. A. Target-blotch culture on 20% cane-juice agar. Concentric rings discernible in center of culture. Aerial mycelium more profuse on the outer portion of the culture. B. Culture of target-blotch fungus on 10% cane-juice agar. Concentric rings are very faint in center of the culture, but somewhat plainer and more numerous on the outer portions. Outer edges somewhat ragged. C. Target-blotch culture on 5% cane-juice agar. Note the absence of concentric rings and the irregular, ragged appearance of the outer edges of the culture.

Growth in 10% Cane-Juice Agar (*Fig. 5, B*). The mycelial growth on this medium is less profuse than on either corn meal or 20% cane-juice agar. The centers of the cultures usually are thin and lie close to the agar surface. Fringes of aerial mycelium, however, may be seen on the outer portions of cultures. Concentric rings usually are present but in most cases are somewhat indistinct except on the outer portions of the cultures. Conidia are produced abundantly over most of the culture area but are more numerous on the outer portions.

Growth in 5% Cane-Juice Agar (*Fig. 5, C*). The type of growth on this medium differs markedly from that on the other agars. The rate of growth from the center is rather slow and the mycelium lies close to the agar surface. The color of the mycelium is gray to dark brown. Rings are faintly discernible in some cultures and absent in others. The mycelium grows out from the center in all directions and forms a dark brown to densely black ring around the outer edge. This ring is caused by the production of numerous conidia on the peculiar hyphal proliferations previously described. From different points on the outer edges of this black ring, single mycelial strands grow out a short way and begin to branch from

a certain point or from several points along their sides. As these little branching points or growth centers continue to spread, they appear to the unaided eye as little individual growths or cultures. Under the microscope, however, the single mycelial strand connecting them to the body of the main culture can be seen very plainly. The mycelial growth of these little centers is usually made up of gnarly proliferations similar to those observed around the edges of the main culture. As the mycelial growth continues to spread, these little growth centers are soon connected to the other part of the culture. From the outer edges of these new developments other strands grow out and start new growths. This method of spread gives the cultures a ragged and peculiar appearance, not observed on the other media used in these experiments. The type of growth is shown very plainly in figure 5, C. In a few cases the outer edges of cultures growing in 10% cane-juice agar approached this type of growth but this characteristic was not usually so pronounced as in the 5% cane-juice agar (Fig. 5, B).

The influence of light on the growth of the target-blotch fungus is very pronounced. Figure 6 shows two single-spore isolations of the same age.

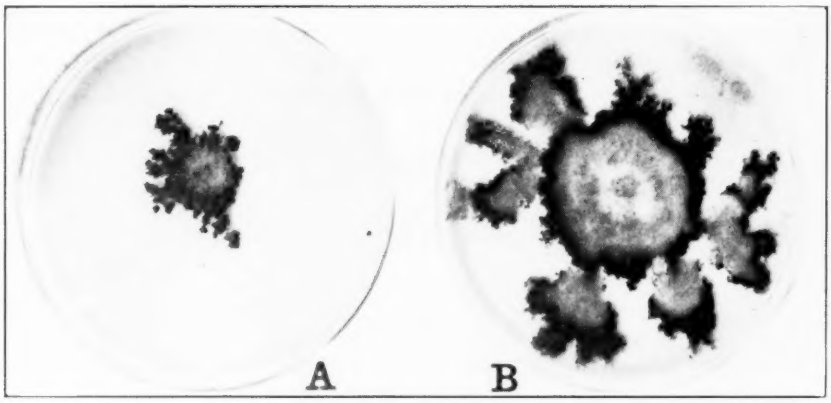

FIG. 6. Two single-spore isolations of the target-blotch fungus. These cultures were plated at the same time and on the same class of agar (5% cane juice). A. Grown in the photographic dark room from which all daylight was excluded. B. Grown in the ordinary daylight of the laboratory.

These were taken from the same culture, plated on the same class of agar (5% cane-juice agar) and grown under the same conditions except that culture A was kept in the photographic dark room from which all daylight was excluded, while culture B was grown in the ordinary daylight of the laboratory. It will be noted from this illustration that the exclusion of light greatly retarded the mycelial development, the size of the culture

grown in the dark being much less than that of the one grown in the light. The production of conidia, however, appears to be stimulated by the absence of light. Spores are produced in innumerable quantities over the whole of culture A, while in B they are most abundant on the black portions around the edges of the culture. Cultures on other classes of media treated in the same way showed a marked difference in the type of resultant growth.

The conidia on the different media are very similar, differing slightly in size and density of color. They are typically light brown to dark brown, depending on the medium on which they are grown. Spores taken from diseased cane leaves usually are light brown.

Microscopic examinations of spores from target-blotch, eye-spot, and brown-stripe cultures show the target-blotch conidia to have a color somewhat darker than the eye-spot spores but considerably lighter than the conidia from the brown-stripe cultures.

The shape of the target-blotch spores is typically slightly curved, the curve being more pronounced on one side due to the bulge of the spore. The thickest part usually is toward one end rather than in the center of the spore (Fig. 3, A).

Germination is typically from the polar segments. The germ tubes usually begin branching very close to the end of the spores (Fig. 3, B). No instance has been observed where germination took place from the intermediate segments. The germination of the spore and growth of the mycelium are very rapid. Under favorable conditions target-blotch spores will begin to germinate within two hours after they have been plated and after twelve hours will have made considerable mycelial growth.

The results from several hundred spore measurements show the average size of the target-blotch spores to be 15.4 $\mu \times 76.2\ \mu$. According to Faris (4) the average measurements of the eye-spot and brown-stripe spores are 12.7 $\mu \times 69\ \mu$ and 17 $\mu \times 84\ \mu$, respectively. A comparison of these measurements shows that the target-blotch spores are larger than the eye-spot but smaller than the brown-stripe conidia.

INOCULATION EXPERIMENTS

The first inoculations from isolated material, as stated elsewhere in this report, were made on vigorously growing, young canes. These inoculations were made by spraying a suspension of the spores in distilled water on leaves of the young plants with a small atomizer. After about two days numerous small red specks were observed on the sprayed leaves. There were no indications of the characteristic rings or blotches of the disease. Results from later experiments suggested this to be due to the rapid growth

of the young canes and to the high temperatures, factors which are unfavorable to the development of the fungus. No further work was done until December, 1928, when new inoculation experiments were started. Several plants of the C 760 variety were inoculated with spores from cultures of single-spore isolation. About twenty-four hours later numerous small red specks appeared on the unrolled leaves. Some of these spots grew together, forming larger spots or blotches but no target blotches developed. On the spindle rolls the infection appeared as small specks, similar to those on the unrolled leaves, but in a short time these small centers of infection became larger and the affected tissues somewhat sunken. Mottled red rings developed around the spots and, as the infection advanced, these red mottled zones became necrotic and changed to a brown straw-color, forming the concentric or zonate rings previously described. On these plants, as on the infected plants observed in the field, the rings continued to form until the leaves became separated from the leaf roll. In most cases the fungus penetrated to the center of the leaf roll and rings were formed on all the leaves present in the spindle roll at the time.

The fungus was reisolated from the infected plants and grown in pure cultures. Spores from these cultures were sprayed on the leaves of several plants of the following varieties: C 47; C 49; C 69; C 760; PR 560; PR 551; SC 12/4; Cristalina; FC 306; D 109; BH 10/12; and Badila. Other plants of these same varieties sprayed with distilled water alone served as checks. After about two weeks target blotches were showing on all of these varieties except FC 306 and D 109. The checks remained healthy. The Cuban and Porto Rican seedlings appeared to be most susceptible. On these varieties the typical target-blotch symptoms developed very readily, but only on the leaf rolls. The results from these and former experiments seemed to indicate that the concentric rings were formed only when infection took place on the leaf roll. To determine this, inoculations were made as follows: Several plants of the C 760 variety were inoculated by spraying the spores on the open leaves only, care being taken to prevent any of the inoculum falling on the leaf rolls. Several other plants of the same variety were thoroughly sprayed on both the open leaves and the leaf rolls. Within a short time the incipient stages of the disease could be seen on all the inoculated leaves of both sets of plants, but the uninoculated spindles of the first set remained free of infection. About ten days later typical target blotches developed on the inoculated spindles of the second set of plants but the spindles of the first set continued free of the disease. No rings formed from any of the inoculations on the unrolled leaves. The results from these and similar experiments performed later show definitely that the rings develop only when infection takes place on the leaf roll. The fungus apparently cannot spread in the tissues of the open leaves.

A mass of fungus mycelium can usually be seen between the layers of leaves in the infected leaf rolls and the spread of infection in the leaf rolls with the subsequent formation of concentric rings appears to be due to the spread of the mycelial hyphae between the leaf surfaces rather than to the advance of the fungus within the leaf tissues. This spread of infection apparently takes place as follows: the mycelial growth develops around the

FIG. 7. Two young cane plants infected with target blotch. Showing method of growing and inoculating plants under strictly aseptic conditions.

edges of the diseased spots between the leaf surfaces and sends haustoria into the healthy cells. As these cells become infected, a mottled red ring is formed which later becomes necrotic and brown. The hyphae of the mycelium then spread across this necrotic ring and send haustoria into the surrounding healthy cells with the subsequent formation of another brown ring. This process of ring development continues as long as the leaves remain in the leaf spindle, but ceases when the leaves unroll due to the drying out and collapsing of the mycelial strands on becoming exposed.

In order to obtain infection under more uniform conditions inoculations were made on plants grown in tall glass cylinders or jars under strictly aseptic conditions. This method of growing and inoculating plants was worked out by Faris (4) and used in his experiments with the eye-spot and brown-stripe diseases. The method is described as follows: the tall glass cylinders were filled to a height of three or four inches with clean sand moistened with a culture solution suitable for the growth of the cane. The jars were stoppered with cotton plugs, the tops wrapped with wrapping paper, and the whole sterilized in a large autoclave at 25 lbs. steam pressure for five hours. The jars were then brought to the laboratory and planted with single-eye seed pieces of the variety C 760, the seed pieces having first been sterilized for 15 minutes in a 1:1000 solution of mercuric chloride. They were covered with sand from smaller cylinders, sterilized along with the larger cylinders. When the cane germinated and had reached a height of six to eight inches they were sprayed with a spore suspension from a single-spore isolation of the fungus. In a short time the leaves of the young plants were thickly covered with small red spots and typical target blotches soon developed on several of the young leaf rolls. Two of the jars with the infected plants are shown in figure 7. The infections produced under these aseptic conditions were free from contaminations of any kind and proved definitely that target blotch is caused by the Helminthosporium fungus.

DISTRIBUTION AND SEASONAL OCCURRENCE

Target blotch was first observed in 1927 on the experiment plots at Central Baraguá. Since that time it has been observed at Central Pilar, Central Jatibonico, Central Jagueyal, and Central Carmita, and probably occurs elsewhere in the island. During the fall and winter of 1927 and 1928 it was found to be rather prevalent on most of the varieties at this station. Some of these varieties were severely attacked, while others were only lightly infected. Usually the spots or blotches are found in the locality of Central Baraguá about the 10th to 15th of December. As the dry, cool weather continues the attacks of the fungus become more severe and widespread. The cool, dry weather and the slow-growing canes at this time

of the year seem to favor the development and spread of the disease. With the coming of the warm, wet weather in the spring the disease gradually disappears except, perhaps, where the old blotches from earlier infections may still be seen.

VARIETAL SUSCEPTIBILITY TO TARGET BLOTCH

Observations during the past two years show that very few of the varieties in the varietal test plots at this station are immune from target blotch. Some of these varieties are highly resistant and show very little effect from the disease, while others are rather severely attacked. On the resistant varieties only a few target blotches may be seen, infection for the most part showing as very small red spots on the unrolled leaves. The susceptible varieties, however, usually show numerous blotches and in a great many cases the tops are killed. Certain of the Cuban and Porto Rican seedling canes are most susceptible but fortunately these canes are not now being grown commercially in Cuba. The newly-introduced POJ varieties, POJ 2883, POJ 2878, POJ 2727, POJ 2725, POJ 2722, POJ 2714, and POJ 36, and CO 281, CO 213, and H 109 are highly resistant to the disease. No typical target blotches have been observed on any of these varieties. Cristalina, Santa Cruz 12/4, and BH 10/12 become infected very readily but usually with slight resulting damage.

The following table gives the relative susceptibility of cane varieties to target blotch as observed on the varietal test plots at this station during the past two winters.

It will be noted from the above table that a great many varieties of sugar cane are susceptible to the attacks of the target-blotch fungus. This table, however, is by no means complete, for there are many varieties which we have had no opportunity to observe in connection with the disease. Only those susceptible varieties, now being grown on the variety test plots at this station, are listed in the above table. Several of the varieties which now appear to be highly resistant to the disease might, under varying conditions, prove more susceptible if observed over a longer period of time. The losses from target blotch on these susceptible varieties usually are small due to the fact that the conditions most favorable for the development and attacks of the fungus do not develop until the canes are well on toward maturity. However, under favorable conditions and on new and perhaps more susceptible varieties, that find their way into the island from time to time, the disease might prove to be more destructive. It therefore is very important that all new varieties be rigidly tested for their susceptibility to this and other diseases before they are distributed as field canes.

TABLE 1.—*Relative susceptibility of cane varieties to target blotch*

1. Varieties severely attacked and on which several typical target blotches usually occur. Infection sometimes results in the death of the entire leaf roll.

C 760	C 145	C 28	PR 724	PR 545
C 228	C 33	C 14	PR 551	HE 45

2. Varieties on which several blotches were observed but whose tops usually were not killed.

C 350	C 31	PR 561	SC 12/4
C 251	C 30	PR 540	BH 10/12
C 69	PR 719	PR 492	B 1809
C 40	PR 700	Cristalina	Yellow Bamboo

3. Varieties on which only a few cases of infection were observed.

C 45	C 24	C 14	PR 449
C 34	C 23	PR 543	PR 417
C 27	C 20	PR 460	PR 260

4. Varieties on which infection is usually very mild. Only a few typical blotches observed.

C 588	C 67	C 47	PR 707	B 67
C 576	C 63	C 46	PR 704	HVR 6307
C 502	C 62	C 45	PR 701	HVR 6159
C 491	C 61	C 44	PR 700	HVR 5039
C 483	C 60	C 43	PR 443	HVR 4124
C 338	C 59	C 42	PR 409	Badila
C 291	C 56	C 17	PR 329	Negrita
C 220	C 55	PR 724	PR 260	Yel. Caledonia
C 145	C 54	PR 719	PR 209	Baraguá 2
D 74	C 50	PR 712	B 3412	BSF 1248
D 108	C 49	PR 709	B 1753	
			B 306	

SUMMARY

1. The symptoms of target blotch are described and illustrated.
2. Isolation and inoculation experiments proved the causal organism to be a species of Helminthosporium. The identity of the species, however, has not as yet been determined.
3. The growth characteristics of the fungus in plate cultures are described and illustrated. The mycelial development on certain media presents some very interesting studies.
4. The attacks of the fungus are most severe on the more mature canes during the winter season.
5. Many varieties of cane are shown to be susceptible to the attacks of the fungus.

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EXPLANATION OF PLATE I

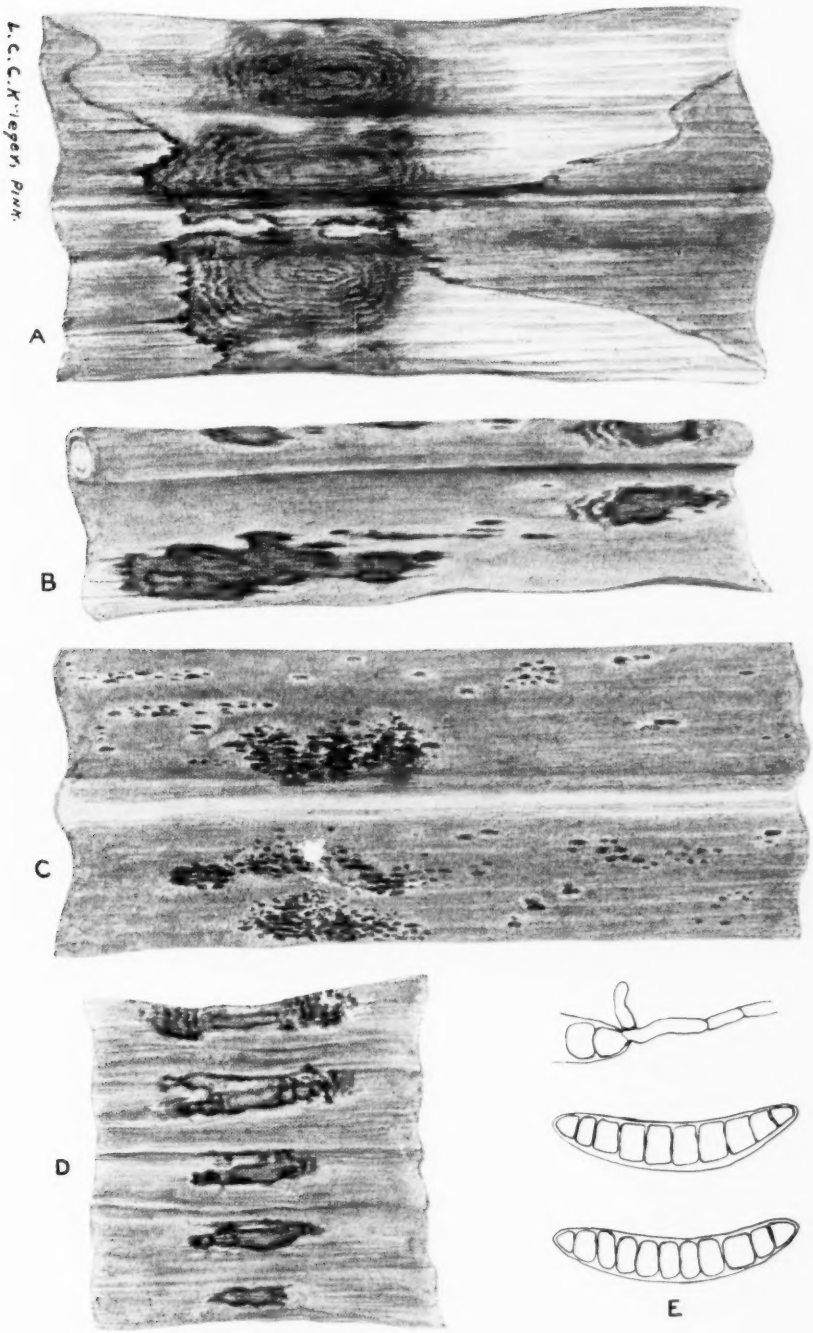
A. A typical target blotch on sugar-cane leaf showing numerous concentric rings.

B. Early stages of target-blotch infection on a leaf roll of sugar cane showing the mottled red rings surrounding the diseased spots and the shrunken condition of the infected tissues.

C. Small infection centers of target blotch on an unrolled leaf of sugar cane. These little infection centers do not enlarge on the unrolled leaves.

D. A section of a young leaf of sugar cane unrolled to show the penetration of the fungus through the successive layers of the rolled leaf.

E. Inked-in photographs of target-blotch spores, × 400.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SUGAR-BEET YELLOWS CAUSED BY FUSARIUM CONGLUTINANS VAR. BETAE

DEWEY STEWART

An unreported disease of sugar-beet roots has been found to occur in Colorado, especially in the southeastern portion, commonly known as the Arkansas Valley. The disease may have been overlooked or previously assumed to be a peculiar manifestation of root rot due to one of the organisms known to be involved in that disease complex. The symptoms of this disease are very distinct, however, and should not have been confused with other diseases of beets. Furthermore, the causal organism as well as the symptoms of the disease has not been described, so it seems probable that a new disease of sugar beets has appeared whose present and potential importance warrant a report.

The name yellows, which fairly well describes the striking appearance of the diseased plants and which has been used for similar diseases of cabbage and celery, is applied to this disease.

SYMPTOMS

Leaf Symptoms. The most characteristic leaf symptom is a yellowing of the mature leaves which gradually appears on the younger ones (Fig. 1). As the disease progresses the heart leaves generally show distortions in the form of an inrolling of the edges and a twisting of the apex to one side (Fig. 2). Leaves showing these distortions lose their pliable texture and appear brittle when grasped in one's hand. Wilting apparently does not occur in large plants under natural conditions; however, wilting frequently occurs with little or no yellowing of the leaves when greenhouse plants are inoculated.

The first symptom presented by large leaves is a yellowing of the portion of the leaf blade between the large veins. Within a few days the entire leaf takes on a yellowish to gray color, except the large veins and a narrow border of leaf mesophyll which may remain green. Shortly, the large veins and petioles die and the dead or dying leaves become heaped around the crown of the diseased plant (Fig. 3).

Root Symptoms. Roots of plants with yellows present almost no external indications of a disease; however, if the root is cut across, the grayish to brown discoloration and rot of the vascular system are evident. The vascular tissue of beet roots occurs in concentric rings (Fig. 1). In most cases only a portion, consisting of one or more rings, of the vascular tissue on one side of the root is involved, which explains the frequent occurrence

FIG. 1. Sugar beet affected with yellows caused by *Fusarium conglutinans* var. *betae*. Early stage showing color change in the larger leaves accompanying the vascular invasion and dry rot of the root. Insert B shows a portion of diseased root in which the vascular involvement is pronounced.

of individuals whose leaves on one side show yellowing and, even, distortions, while leaves on the other appear healthy. Eventually all the leaves of an affected plant show symptoms of disease, as the organism spreads laterally, involving other parts of the root.

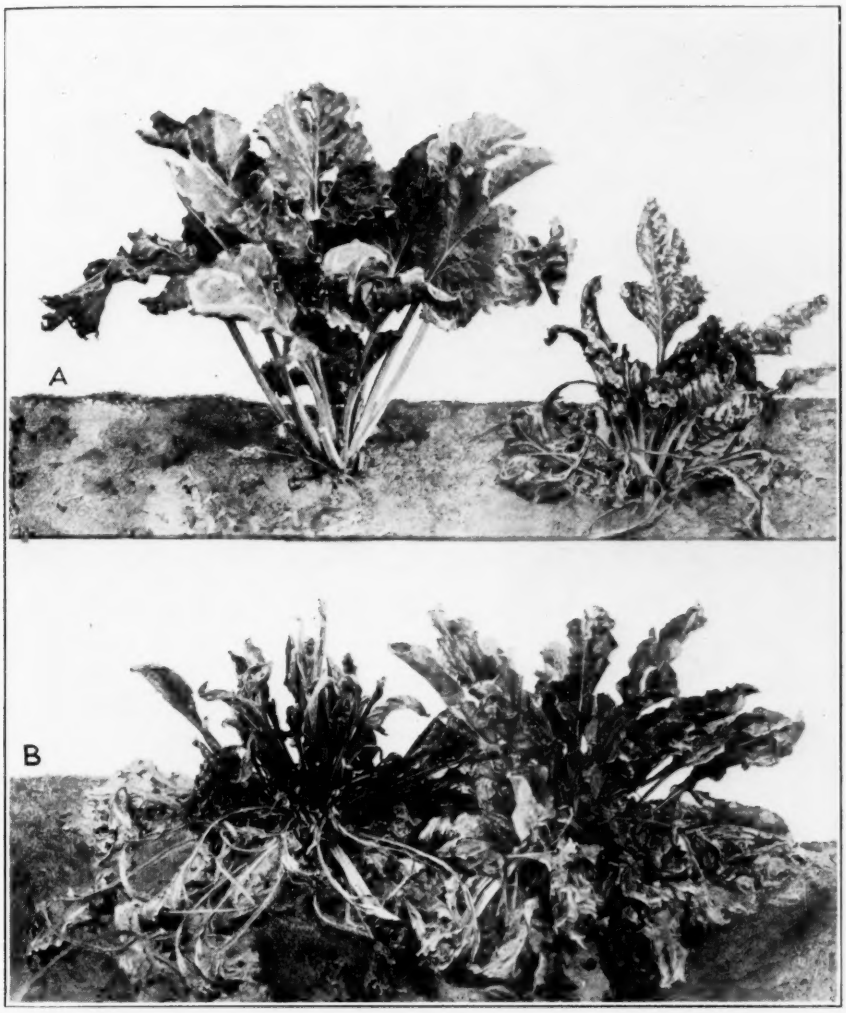

FIG. 2. Normal sugar beets contrasted with those affected with yellows: A, Early-season aspect (diseased plant at right). B, Late-season aspect of the disease showing the diseased plant (left) with only one or two of the mature leaves alive and the heart leaves furled and distorted.

By gradually cutting away a diseased root from crown to tip the path of the fungus invasion can often be traced to the starting-point in a lateral root, usually located 5–8 inches below the surface of the ground. It would seem that the pathogene gains entrance through small lateral roots rather than wounds, and the occurrence of the initial infection at certain depths is thought to be related to the prevalence of lateral roots rather than to a restricted location of the fungus in the soil.

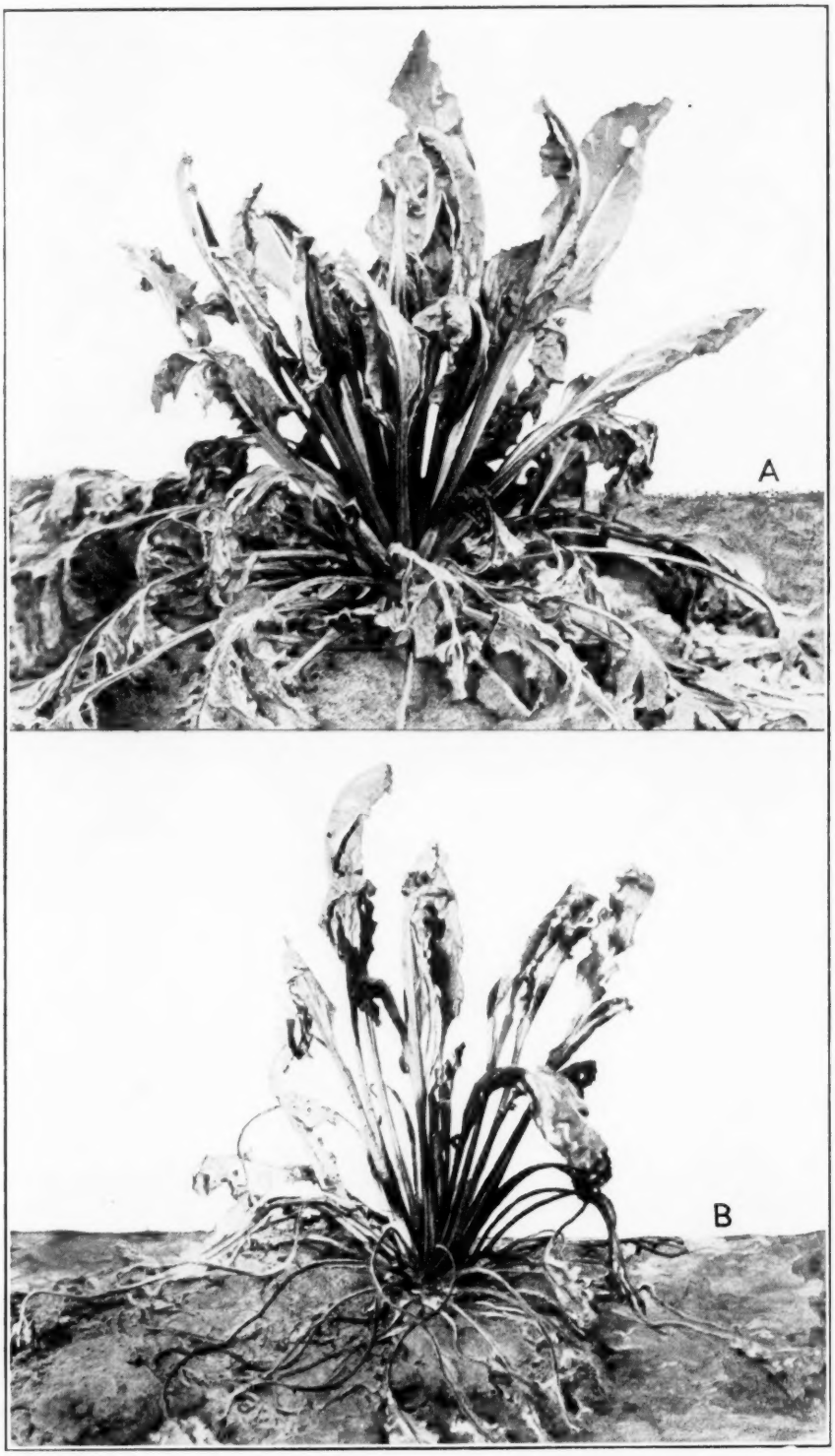

FIG. 3. Sugar beets in late stages of yellows: A, Older leaves dead and the leaves of the inner whorls distorted. B, Older leaves dead, growth of heart leaves completely checked.

Seedling Symptoms. The symptoms manifested by seedlings with yellows may be a typical wilt in which the leaves and cotyledons become flaccid, curled, and dry without ever showing a yellow color. In larger seedlings the leaves may become yellow, duplicating the leaf symptoms of large plants in the field. In most cases after the cotyledons and leaves are diseased the stem remains upright with no apparent injury; however, if the stem is broken the vascular system shows a grayish to brown color. The tendency for the diseased seedling to remain upright, as well as the general absence of external decay, usually distinguishes this disease from ordinary damping off.

IMPORTANCE

The value of beet roots is determined by the weight and the percentage of sugar they contain. Beets low in sugar are discriminated against, due to problems they present during the processes of beet-sugar fabrication, and some companies reserve the right to reject beets testing less than 12 per cent sucrose. This disease has been found to produce a marked effect on the sugar percentage and a depressing effect on the weight of roots.

Analyses were made of healthy and diseased roots in order to determine the influence of this disease upon weight and percentage of sucrose. At the time of collecting the diseased plants, the healthy one growing next along the row, which was produced under similar conditions except for disease, was taken for comparison. Table 1 gives in summary form the results of the determinations from 15 beets of each class.

From these data it is evident that the percentage of sucrose is reduced in the diseased plants even from the first indication of disease. The greater reduction in percentage of sugar is found in the individuals showing the more advanced stages of disease. The diseased roots, judging from the determinations at harvest, show a significant reduction in weight.

Since sugar beets are susceptible to the pathogene from seedling stage to maturity a loss of plants occurs throughout the season in infested fields. The total loss of individuals in a particular field due to this disease has not been determined, yet as many as 1 per cent of the plants have been found diseased at the time of making a survey. Probably more important than the present-day losses is the fact that beet yellows belongs to that increasing group of plant diseases having as their causal agent some species of the genus Fusarium, all of which are very difficult to control.

ETIOLOGY[1]

The fungus causing beet yellows belongs to the genus Fusarium; however, complete sporulation, which would enable one to compare it with

[1] The etiological studies were made while the writer was a graduate student at Cornell University and he wishes to express his appreciation for the facilities of the Department of Plant Pathology.

TABLE 1.—*Comparison of roots from yellowed and healthy plants. Plants collected near Rocky Ford, Colorado—1927. Results given as averages of 15 individual readings*

Time of collecting	Weight (gms.)			Percentage of sugar		
	Healthy	Diseased	Difference	Healthy	Diseased	Difference
Late summer						
Slight disease	552.0 ± 39.16	447.0 ± 22.12	105 ± 47.15	10.36 ± .60	8.20 ± .53	2.16 ± .80
Severe disease	535.5 ± 70.88	418.5 ± 56.50	117 ± 90.64	10.68 ± .39	6.68 ± .29	4.0 ± .48
Just before harvest						
Average	895.9 ± 37.93	353.0 ± 39.59	542.0 ± 54.8	12.00 ± .27	7.67 ± .37	4.33 ± .46

known species of the genus or establish a new species, has not been obtained. The fungus resembles *Fusarium conglutinans* Wollw. more than other Fusaria and is provisionally placed as a variety of this species. On the basis of its morphological and physiological characters the organism has been named *F. conglutinans* var. *betae*.

Isolation of the organism from affected tissue can readily be accomplished, but, since a sugar-beet root offers an excellent substratum to most soil fungi, after its natural resistance is once broken down, precaution must be taken to make sure that only recently invaded tissue is used. In order to make more certain of having the proper disease, when first attempting to isolate the pathogene, only plants showing marked symptoms were used. This material for the most part gave cultures of Fusaria. The prevailing type of culture was characterized by a peculiar cottony white mycelium and its failure to produce a pigment on glucose agar. In a few isolations the Fusarium obtained produced various shades of red pigment. These undoubtedly were secondary invaders, since later inoculations failed to produce disease.

The association of this particular organism was further demonstrated when isolations were made from plants showing the first symptoms of disease. The bit of tissue plated was always taken from the region separating diseased and healthy portions of the root. The results of isolations made from 103 plants in this manner are given in table 2.

TABLE 2.—*Organisms isolated from roots of sugar beets with yellows*

Location	Date 1927	No. roots	No. roots with *Fusarium conglutinans* var. *betae*	Other organisms
Manzanola, Colorado	June 26	7	7	0
Rocky Ford, Colorado	July 8	50	33	5 bacteria 12 no growth
Rocky Ford, Colorado	Aug. 12	21	20	1 Rhizoctonia
Rocky Ford, Colorado	Aug. 23	25	23	2 no growth
	Total	103	83	

Thus, from 103 plants with yellows, there was found associated a particular type of Fusarium in 80 per cent of the roots. If the instances of "no growth" are accounted for as excessive sterilization of the piece of tissue and the bacterial growths as contaminations, there remains only one failure to secure *Fusarium conglutinans* var. *betae* from plants in the early

stages of this disease. Since species of Rhizoctonia are commonly associated with root rots of sugar beets the one isolated is undoubtedly a secondary pathogene or the early symptoms of yellows may have been confused with the disease produced by this Rhizoctonia.

The pathogenicity of *F. conglutinans* var. *betae* was demonstrated by the following method. Healthy sugar-beet plants about 6 weeks old growing in small pots were transferred, with the adhering dirt, to a larger pot of sterilized soil. Some of the pots were inoculated with *F. conglutinans* var. *betae* and within three weeks typical symptoms of beet yellows appeared, while the plants transferred to soil without the organism remained healthy. Tissue platings were made from the small lateral roots as well as the vascular tissue of the main root of these diseased plants and in every case a Fusarium similar to *F. conglutinans* var. *betae* was obtained.

CLASSIFICATION

The Fusarium causing sugar-beet yellows has been grown on many kinds of cultural media, such as stems of sweet clover, red clover, alfalfa, tomato, and alnus, beet petioles, beet and potato plugs, as well as synthetic and vegetable-decoction agars, at temperatures varying from 3° to 33° C. and hydrogen-ion concentration varying from pH 3.7 to pH 9.2, but in no case have macroconidia been produced. The outstanding cultural character of the organism is the absence of pigment on steamed rice, which is characteristic of relatively few species of Fusaria. Wollenweber[1] used the absence of pigment on steamed rice as a basis for separating *Fusarium conglutinans* Wollw. from *F. orthoceras* Appel and Wollw. *F. orthoceras* is a soil saprophyte or a weak parasite, while the orthoceras-conglutinans group contains important pathogenes, such as the aster-wilt[2] organism and the pea-wilt organism recently reported by Linford.[3]

Cultures of the organism causing beet yellows were submitted to C. D. Sherbakoff but, he being unable to obtain macroconidia, stated "that superficially it resembles *F. conglutinans* more than other Fusaria." He pointed out, however, "certain peculiarities by which it apparently differs from *F. conglutinans* or *F. conglutinans* var. *callistephi;* namely, it produces extremely fine ultimate hyphae which often grow in rather characteristic curves— also differs from them in the chlamydospores which in your Fusarium are often observed in fairly long intercalary chains."

[1] Wollenweber, H. W. Pilzparasitare Welkekrankheiten der Kulturpflanzen. Ber. d. Deut. bot. Ges. **31**: 17–34. 1913.

[2] Beach, W. S. The Fusarium wilt of China aster. Rpt. Mich. Acad. Sci. **20**: 281–308. 1918.

[3] Linford, M. B. A Fusarium wilt of peas in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 85. 1928.

Cultures of *Fusarium conglutinans* and *F. conglutinans* var. *callistephi* were secured from G. H. Coons, formerly of the Michigan State College, as well as J. C. Gilman, Iowa State College. These Fusaria along with the one from sugar beet were used to inoculate pots of sterilized soil. The pots were inoculated with a single organism and then equal portions planted to sugar beets, cabbage, and asters. In every case disease occurred only in the species of plants from which the organism was originally secured. The inability of the sugar-beet Fusarium to produce disease in cabbage or aster was further demonstrated by inserting the organism into stems of plants, and no indication of a disease resulted. Likewise, beet roots were inoculated with *F. conglutinans* and *F. conglutinans* var. *callistephi* without the production of disease symptoms.

These tests show that *F. conglutinans* var. *betae* is distinct from *F. conglutinans* or *F. conglutinans* var. *callistephi* in pathogenicity.

Technical Description.—**Fusarium conglutinans** var. **betae,** nov. var.

The microconidia are hyaline or grayish in mass, 2–3 μ wide and 7–12 μ long; continuous except for an occasional septate spore in old cultures; straight to slightly curved. The chlamydospores are globose to ovoid and may be terminal or intercalary, the latter are generally produced in long chains. The mycelium is cottony white with aerial growth abundant. Its most striking cultural characteristic is the absence of pigment production on steamed rice. Pathogenic to sugar beets, producing disease called yellows. Found in commercial beet fields in Colorado.

PHYSIOLOGY

Growth on Media. The organism will grow on almost any vegetative substrate or agar of common formula; however, the abundance of mycelium produced depends upon the amount of soluble carbohydrates present.

Temperature—pH. The influence of temperature and reaction of the medium on the growth of *Fusarium conglutinans* var. *betae* were deter-

TABLE 3.—*Growth of Fusarium conglutinans var. betae agar at different pH and temperature*

	Temperature °C.										
	3	6	9	12	15	18	21	24	27	30	33
pH	Growth in cm.										
3.7	0	0	+	0.9	1.2	2.7	3.1	3.9	3.9	2.0	1.0
4.7	0	0	+	1.4	2.2	3.5	4.2	5.6	5.7	3.2	2.0
5.8	0	0	+	1.8	2.7	4.2	4.8	5.8	5.9	3.6	2.5
7.0	0	0	+	1.5	2.4	3.5	4.5	5.5	5.5	2.7	1.5
8.6	0	0	+	1.2	2.0	3.1	3.5	4.4	4.5	2.2	1.3
9.2	0	0	+	0.7	1.1	2.2	2.8	3.4	3.5	1.8	0.3

mined by growing the fungus on potato-dextrose agar. Enough agar for the entire experiment was prepared in one batch and adjusted to the desired pH as it cooled after sterilization. The agar plates were inoculated by taking small squares from a plate culture previously prepared. The rate of growth was determined by measuring the diameter of colonies produced during a period of 5 days. The values given in table 3 represent the average diameter of growth recorded from three cultures.

The optimum temperature for growth of this fungus on potato-dextrose agar was found to be 24–27° C. (Fig. 4), while the optimum reaction of the agar was on the acid side of the neutral point. The influence of the reaction

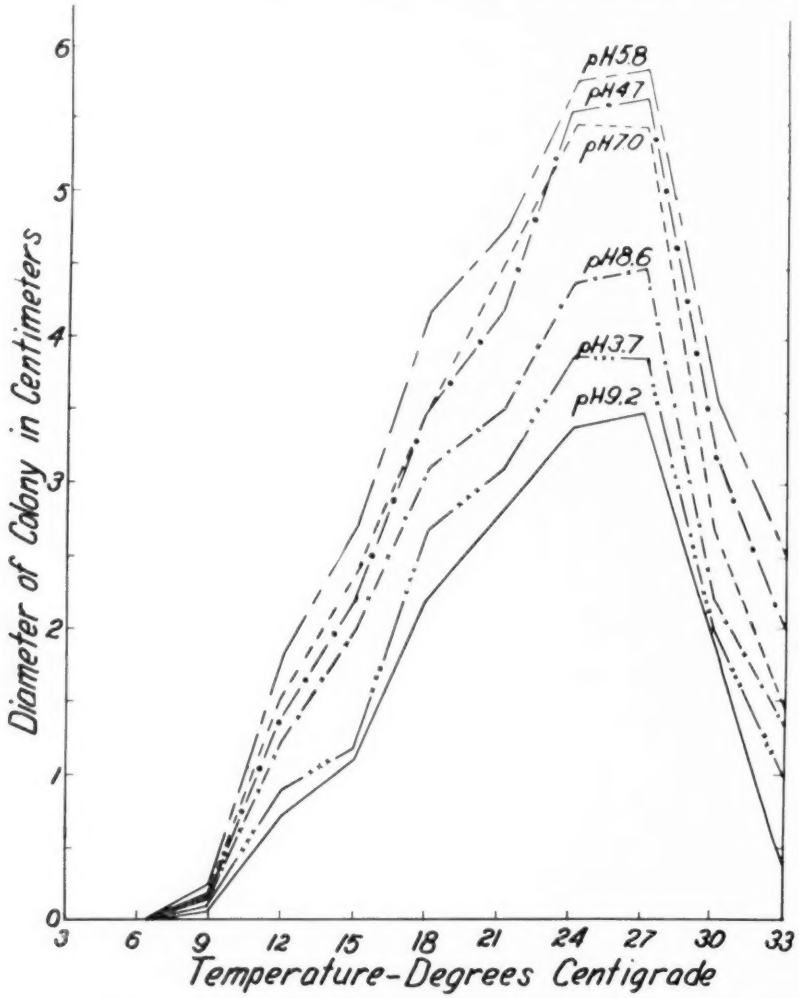

FIG. 4. Growth of *Fusarium conglutinans* var. *betae* on potato-dextrose agar.

of the substratum is of interest, since most soils where the disease has been found are neutral to alkaline in reaction.

VIRULENCE OF THE PATHOGENE

The amount of disease produced in seedlings by *Fusarium conglutinans* var. *betae* was compared with *Corticium vagum* B. and C. (*Rhizoctonia solani* Kuhn) and *Pythium debaryanum* Hesse. The soil, a sandy loam, was sterilized by steaming under 15 pounds pressure for 4 hours. The organisms were grown in large quantities on sterile wheat kernels and transferred to the soil about a week before planting the seed. The seed balls were pasteurized at 60° F. for 10 minutes on three consecutive days to eliminate seed-borne organisms. Fifty seed balls were planted to a 5-inch pot. The seedling counts given in table 4 represent a 14-day period after the plants began to emerge.

Isolations were made from one or more diseased seedlings from day to day and in every case only the organism with which the soil had been inoculated was obtained.

The conditions of this test were very favorable for the production of seedling disease, as demonstrated by the failure of a single seedling to get

TABLE 4.—*Disease produced in seedlings by certain soil-inhabiting pathogenes of sugar beets*

	Pot	Plants emerging		Amount of disease	
		Total	Plants per seed ball	No. plants	Per cent
Check	A	62	1.24	0	0
	B	71	1.42	0	0
	C	66	1.32	0	0
	D	70	1.40	0	0
		67.25	1.345		
Fusarium conglutinans var. *betae*	A	60	1.20	49	81.7
	B	72	1.44	56	77.8
	C	59	1.18	46	78.0
	D	73	1.46	61	83.6
		66	1.320		80.3
Pythium debaryanum	A	5	.10	5	100
	B	18	.36	18	100
	C	11	.22	11	100
	D	10	.20	10	100.0
		11	.22		
Rhizoctonia solani	A	0	0	0	0
	B	0	0	0	0
	C	0	0	0	0
	D	0	0	0	0

above the surface in the soil inoculated with *Rhizoctonia solani*. The organism was readily isolated from the seed balls. In the case of *Pythium debaryanum* a few seedlings emerged but they were all diseased before the end of the 14-day period. It is interesting to note that the total of plants emerging in the soil inoculated with *Fusarium conglutinans* var. *betae* was very similar to that in the sterilized soil; however, within 14 days 80 per cent of the plants had become diseased. From these data it is evident that *F. conglutinans* var. *betae* can be classed as a virulent pathogene of sugar beets but a slower-acting organism than *R. solani* or *Pythium debaryanum*.

Plants affected. The organism causing beet yellows is known to produce disease only in beets. It has occurred in fields used for variety testing but not in sufficient amounts to enable one to ascertain varietal differences in susceptibility. Various crop plants, such as lima beans, string beans, cantaloupes, cucumbers, alfalfa, cabbage, and asters, grown in the region where the disease is known to occur, were planted in infested soil or inoculated by inserting the organism into the stems of plants; however, none of the plants so treated became diseased.

SUMMARY

A new disease of sugar beets has been found to occur in the beet fields of Colorado, which is characterized by the production of a yellowing of the leaves and a gray dry rot of the vascular system of the roots.

It is important due to the marked reduction in the percentage of sugar and weight in the diseased plants as well as the loss of individuals during the summer.

The pathogene appears to be new to literature and is here provisionally named *Fusarium conglutinans* var. *betae*.

The optimum temperature for growth of the pathogene on potato-dextrose agar was found to be 24–27° C. The greatest growth occurred at pH 5.8 of the hydrogen-ion concentrations used.

The pathogene can be classified as a virulent one on seedlings, but its lethal effect is delayed as compared with that of *Rhizoctonia solani* or *Pythium debaryanum*.

OFFICE OF SUGAR PLANTS,
BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

ALFALFA DWARF, A HITHERTO UNREPORTED DISEASE[1]

J. L. WEIMER[2]

INTRODUCTION

For the last few years alfalfa growers in California, like those in many other States, have experienced difficulty in maintaining their stands of alfalfa for a satisfactory period of years. This fact was brought to the writer's attention in 1927 by B. A. Madson of the University of California. A study of diseased alfalfa roots sent by Professor Madson to F. R. Jones and the writer led to the conclusion that the trouble in some parts of California was due to the bacterial wilt caused by *Phytomonas insidiosa* (McC.) Bergey *et al.* However, diseased plants from other parts of the State, although exhibiting somewhat similar symptoms, did not appear to have the wilt bacteria present. Aside from difficulties primarily due to cultural and soil factors, which it is not the purpose of this paper to discuss, further study by the writer has shown that, the shortening of the life of the alfalfa stands in California can be attributed to two causes. Throughout the San Joaquin Valley, at least from Bakersfield to Modesto, bacterial wilt is largely responsible for the premature depletion of the alfalfa stands. This disease has been reported also from the Sacramento Valley. On the other hand, wilt is rarely found south of the Tehachapi Mountains, but another disease possessing some of the characteristics of wilt is very prevalent and destructive there. It is the purpose of this paper to give a brief preliminary report on this latter disease, which, so far as the writer is aware, has not been described hitherto.

THE DISEASE

Name. Plants affected with the disease under discussion gradually become smaller until they finally succumb without presenting any other striking top symptom; hence the name "alfalfa dwarf" is suggested. To the writer's knowledge the only name that has been applied to this trouble by growers is "little leaf." However, since the stems also become short and slender the name "dwarf" seems more descriptive of the symptoms.

History and Geographic Distribution. When or where the disease originated is not known. Many farmers state that until about 10 or 12

[1] Cooperative investigations between the California Agricultural Experiment Station and the Office of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Senior Pathologist, Office of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

years ago they had no difficulty in maintaining satisfactory stands of alfalfa for 8 to 10 years or longer, whereas at the present time a stand is seldom worth maintaining for more than 3 years in sections where the disease is most destructive.

In a discussion of the status of alfalfa in the Riverside area, Nelson and associates[3] state that the yields of alfalfa on sandy land frequently begin to decline somewhat after the fourth year and that the crop often becomes unprofitable after the seventh or eighth year. Since no mention is made of a decline of yields on the heavier soils, it seems quite probable that no general trouble such as exists at the present time was being experienced then (1915). A survey of the alfalfa sections of southern California, made by B. A. Madson during the summer of 1927, showed that at that time the stands remained productive for only two to four years.[4]

No evidence of the existence of this disease outside of southern California is at hand. However, no extensive survey has been made to determine the limits of its distribution.

Economic Importance. Present information indicates that the dwarf disease is largely responsible for the short-lived stands of alfalfa in southern California. In some sections the disease limits the life of the stand to two years, while in others a satisfactory crop is harvested for a period of four years or longer. Certain regions, such as the Mojave Desert section of San Bernardino County, the Hemet Valley, and a few other places, suffer little or not at all from the disease. In some of these sections the disease seems very limited in its distribution, while in others it is widespread but apparently held in check largely by cultural conditions.

The disease not only shortens the life of the stand but cuts down the yield materially, especially during the latter part of the last year of the stand. Fields have been seen in which nearly every plant was affected, the stand and the yield being greatly reduced. No doubt this disease causes a loss of thousands of dollars annually.

The disease may be distributed more or less uniformly in the field, or it may be limited to certain patches. The latter is more commonly the case, at least in the early stages. Most frequently it appears first along irrigation ditches or where the soil moisture is highest and spreads from these areas until most or all of the field is involved.

Symptoms. The earliest stages of alfalfa dwarf can not be detected by the aboveground symptoms, since the disease is already well advanced

[3] NELSON, J. W., R. L. PENDLETON, J. E. DUNN, A. T. STRAHORN, and E. B. WATSON. SOIL SURVEY OF THE RIVERSIDE AREA, CALIFORNIA. U. S. Dept. Agr., Bur. Soils Field Oper. 1915 (Rpt. 17.): 2367–2450. 1919.

[4] MERRILL, E. D. REPORT OF THE DIRECTOR OF THE AGRICULTURAL EXPERIMENT STATION. Calif. Agr. Exp. Sta. Ann. Rpt. 1926/27. 1927.

in the root before it becomes evident in the top. The first signs of the disease in the tops, are a shortening of the stems and a slight reduction in the size of the leaves. Blossoming is often retarded or inhibited. After each cutting the stems of affected plants become shorter and slenderer and the leaves smaller. Likewise, fewer new buds are developed each time, resulting in a gradual diminution of the number of stems. Usually, no chlorosis or other color change occurs in the leaves or stems until the last few stems die. In the final stages of the disease only one or at most a very few stems are produced, and these reach an ultimate height of but a few inches. These stems remain upright and for the most part turgid until death ensues. Not infrequently the leaves of diseased plants have appeared to be of darker green than those of neighboring healthy plants. The leaves of affected plants are not mottled, crinkled, or deformed, although commonly they are somewhat rounded at their apices, resembling more closely the basal than the terminal leaves of healthy plants. Although not of very common occurrence, wilting of the tips of the stems may occur in the later stages of the disease even in quite wet soil. The stems of diseased plants usually are reduced more or less uniformly in size. (Fig. 1.)

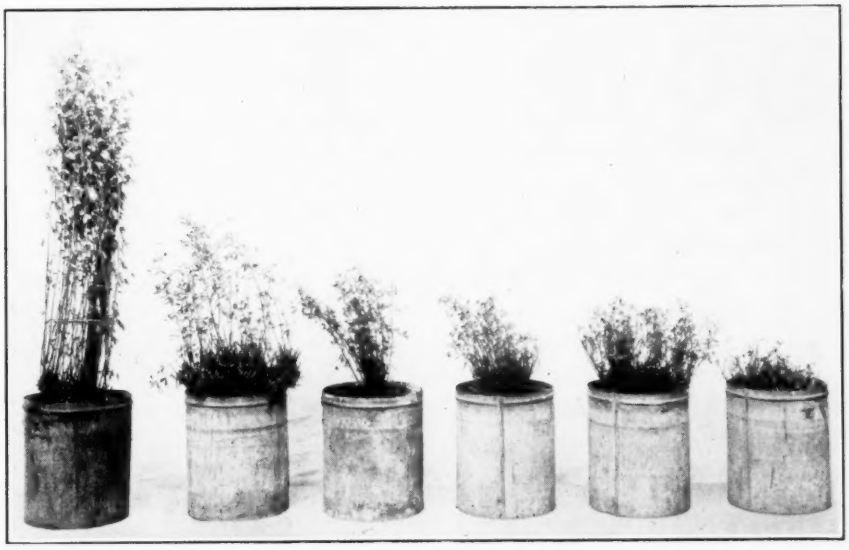

FIG. 1. Alfalfa plants of the Chilean variety affected with the dwarf disease. The healthy plant at the left is of the same age and from the same part of the field as the other plants, which are diseased. Note especially the slender stems of the diseased plants as compared with those of the healthy, and the gradual diminution in height. The plants were removed from the field just before being photographed.

The first evidence of this disease in the root thus far observed is a small yellow streak in the wood, apparent only when the bark is removed. This streak, which may be from a few millimeters to several centimeters long by a few millimeters wide, may occur anywhere in the root, especially in the upper foot of the taproot. As the disease develops, the yellowing spreads until it eventually involves the entire circumference of the root. As far as observed, yellowing is never present in the bark unless secondary infection has begun. When the root is cut across, the yellow color is found in the outermost part of the woody cylinder just beneath the bark. This discolored tissue forms a definite ring or band, which is narrow at first but becomes wider as the disease develops, until at the time the plant dies the root is frequently discolored throughout its entire diameter. How much of this discoloring is due to secondary causes is not known. Plants may die while the band of yellow tissue is comparatively narrow, but in this case it extends to the cambium. This condition is commonly seen during the hot summer months when the plants are dying most rapidly. During the winter and early spring months the yellowing usually does not extend to the cambium but is buried beneath a thin layer of new xylem, indicating that the disease it not active during the winter months. The xylem of the secondary and tertiary roots also becomes yellow, and death ensues. Sometimes small roots are formed near the crown, which support a more vigorous top growth than seems possible, considering the condition of the main taproot. The yellowing extends into the main divisions of the crown and into the base of the green stems, but thus far it has not been found to go very far up into the latter.

The yellowing in the roots of plants affected with dwarf, being much lighter in color, is distinctly different from that produced by the Fusarium wilt described by Weimer.[5]

Plants with slender stems and small leaves resembling those of plants affected with the dwarf disease have been seen in fields suffering from drought or from some soil deficiency, but such plants do not show the yellowing in the roots. The roots as well as the tops of such plants are usually small, whereas the roots of plants affected with dwarf frequently are the largest and most vigorous in appearance of any others in the particular area in which they are found.

COMPARISON OF ALFALFA DWARF AND BACTERIAL WILT

Since the symptoms produced by the dwarf disease and by bacterial wilt are so similar that it is often difficult for one not familiar with both diseases to tell them apart, it seems desirable to compare rather definitely

[5] WEIMER, J. L. A WILT DISEASE OF ALFALFA CAUSED BY *Fusarium oxysporum* VAR. *medicaginis*, N. VAR. Jour. Agr. Res. 37: 419–433. 1928.

the symptoms of each. The following points should be helpful in distinguishing between these two diseases.

(1) Both wilt and dwarf cause a decided stunting of the tops of the plants as the diseases progress, although neither produces evident top symptoms in the earliest stages.

(2) In both diseases the stems become fewer, shorter, and more spindling after each cutting, until only a very few stems are produced, and these eventually die.

(3) Bacterial wilt causes stunted and abnormally shaped leaves that are usually paler in color than those of healthy plants. In the dwarf disease the leaves become quite small but remain practically normal in shape and color until the death of the plant.

(4) Both diseases may produce wilting under certain conditions, but this is not a constant or very conspicuous symptom of either disease.

(5) The root symptoms produced by the two diseases are very similar. The earliest stage that can be detected in roots affected with either disease is a slight yellowing of the wood just beneath the bark. This yellowing, which results largely from the formation of gum in the vessels, spreads until the entire active part of the xylem is more or less completely involved. The reddish-brown lesions in the bark and wood of roots affected with wilt, described and illustrated by Jones and McCulloch,[6] have never been seen in roots affected with dwarf.

(6) In case of doubt the presence of the bacteria in the vessels of the roots of plants affected with wilt can easily be demonstrated in freehand sections by Gram's stain. No bacteria will be seen in similarly treated sections of roots having the dwarf disease.

SUMMARY

A brief preliminary description of a hitherto undescribed disease of alfalfa occurring in southern California is given. The symptoms of this disease are quite similar to those of bacterial wilt; hence the similarities and differences between the two are pointed out.

[6] JONES, F. R., and L. MCCULLOCH. A BACTERIAL WILT AND ROOT ROT OF ALFALFA CAUSED BY *Aplanobacter insidiosum* L. McC. Jour. Agr. Res. 33: 493–521. 1926.

DIAPORTHE BLIGHT OF LARKSPUR

FREDERICK A. WOLF

Larkspur, *Delphinium ajacis* L., within the United States, is subject to several diseases two of which are more or less well known, sclerotial blight caused by *Sclerotium delphinii* Welch (5, 3), and bacterial leaf spot caused by *Bacterium delphinii* (E. F. S.) Bryan (1). The former appears as a decay of the cortical portion of the stems at or near the ground level, eventuating in a girdling and death of the plants. The presence of sclerotia in a mycelial weft near the base of the stem constitutes a characteristic sign of this disease. The latter may be recognized by the presence of dark brown to blackish spots on the leaves and streaks on the stems.

Another disease, apparently undescribed, and one that may be appropriately designated "Diaporthe blight," was first observed in the vicinity of Durham, N. C., during the spring of 1929. Attention is first directed to this disease because of the fact that the lower leaves of plants which have reached the flowering stage become brown and dry and remain attached. Closer examination shows that brown lesions occur near the base of the stems. By the time that these lesions have developed to the extent of completely girdling the stems, the entire foliage will have gradually withered and become dry. Eventually the lesions extend upward several inches above the surface of the soil and downward into the root system. Scattered dark pycnidia are present within the stems (Fig. A) and within the petioles and leaf blades of the lowermost leaves before the plants have succumbed, and new pycnidia continue to be formed for a considerable period thereafter. The crown and uppermost roots are enveloped in a cottony weft of mycelium during rainy periods.

Toward the close of the growing season scattered pycnidia appear upon the capsules. Such capsules are in some cases blighted and fail to mature and in others apparently normal seed are borne in affected capsules. The seed from diseased capsules, in all likelihood, serve as a means of initiating this blight in new localities.

Microscopic examination shows that the spherical to ellipsoidal pycnidia which measure 100 to 150 μ are wholly immersed within the tissues. They are of the Phomopsis type, possess a wall of dark cells, and contain a single locule from which the conidia are exuded by a pore. They bear two kinds of hyaline conidia, alpha conidia (Fig. B, 3), which are capable of germination, and beta conidia or stylospores (Fig. B, 2), which have never been noted to be capable of germination. The former are developed less abundantly both on larkspur stems and in culture. They are oval and

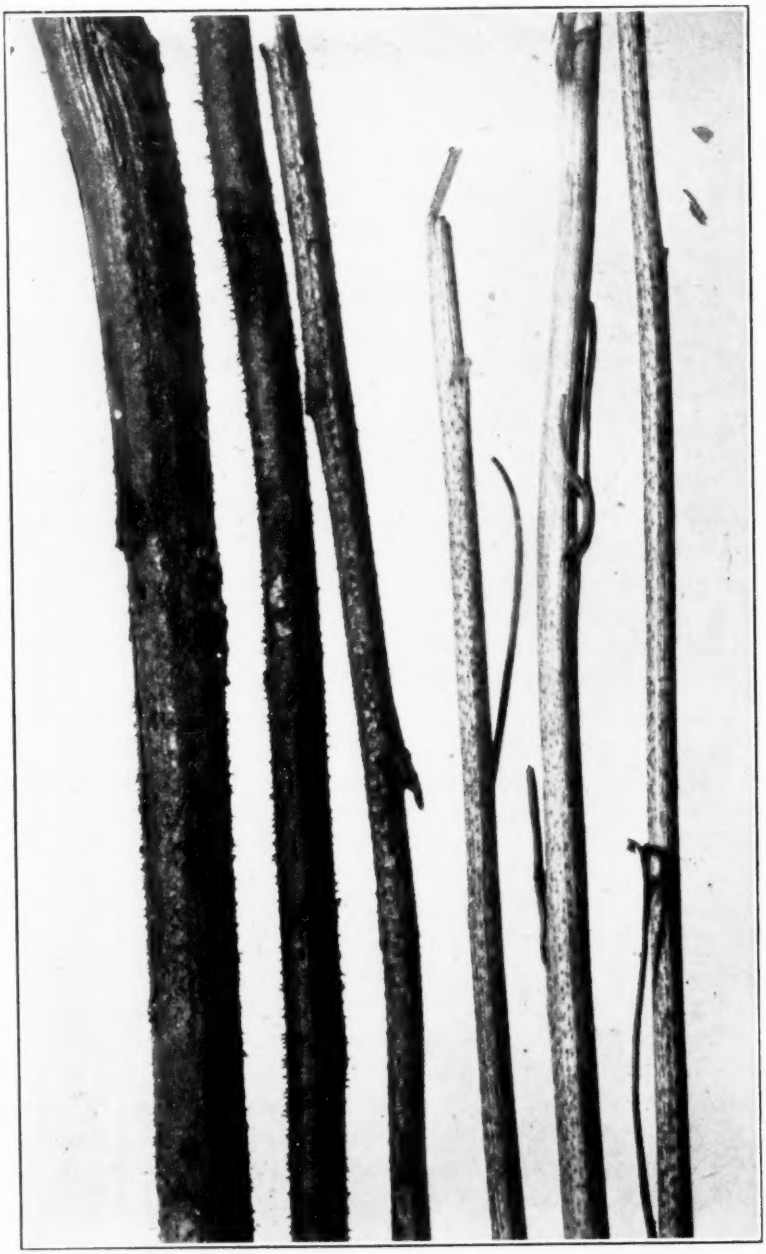

FIG. A. At the left, three stems of larkspur bearing the conidial stage of *Diaporthe arctii*, and, at the right, three bearing the ascigerous stage.

measure 8–10 by 3.54 μ, while the latter are thread-like and hooked or curved and measure 20–30 by 0.75 μ.

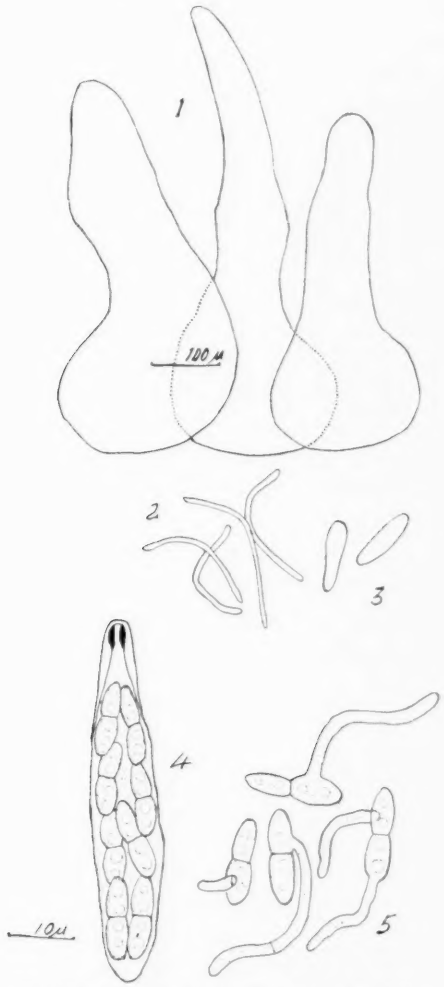

FIG. B. 1. Perithecia of *Diaporthe arctii* in outline. 2. Beta conidia or stylospores. 3. Alpha conidia. 4. Typical ascus showing arrangement of ascospores and apical pore. 5. Germination of ascospores on agar after 15 hours.

A bundle of blighted stems was collected during May, 1929, and allowed to remain out of doors in contact with the soil until the following March. In the interim the surface of the stems had been blackened and a perithecial stage of the Diaporthe type had developed (Fig. A). There is no well-defined ventral zone. The perithecia occur singly, are sunken within

the substratum, and extend to the surface by means of thick, elongated beaks 300 to 400 μ long (Fig. B, 1). The diameter of their basal portion is 200 to 225 μ and of their beaks 75 to 100 μ. The asci are 45–50 by 9–10 μ, with a refringent apical pore. The ascospores are hyaline, one-septate, guttulate, are arranged biseriately, and measure 10 to 12 by 3 to 3.5 μ (Fig. B, 4).

Isolations were made by taking advantage of the fact that the ascospores are forcibly expelled. Stems bearing perithecia were placed on moist blotting paper in the tops of inverted agar plates. Within a few hours great numbers of the ascospores were found to have adhered to the surface of the agar above. When the ascospores were transfered to tubes of potato agar or to sterilized larkspur stems, the colonies which developed consisted of a loose white mycelium. After a period of about two weeks, Phomopsis pycnidia had formed sparingly in these colonies.

Additional evidence of the genetic connection of the perithecial and conidial stage and, at the same time, of pathogenicity was obtained by series of inoculations. In one series the inoculum consisted of drops of water containing macerated perithecia; in another, a suspension of conidia from pure cultures isolated from ascospores. Small brown lesions were apparent on all inoculated plants 7 to 8 days after inoculation, and Phomopsis pycnidia had formed within the succeeding 10-day period. The larkspur-blight organism is, therefore, another Diaporthe of the group whose conidial stage is parasitic and whose perithecial stage develops on dead or decaying parts, typified by such well-known pathogenes as *D. phaseolarum* (C. & E.) Ell., *D. batatatis* Harter and Field, and *D. citri* (Fawcett) Wolf.

The question of the identity of the larkspur-blight fungus revolves around whether one should regard it as specifically identical with previously described forms on the basis of morphologic resemblance to such species, or whether one should emphasize its distinctness on the basis of suscept relationships. Many species of Diaporthe are known to be limited to closely related suscept species, a fact which has served as a means of identification of species which are morphologically alike or are very similar to previously described forms on other kinds of plants. Recently, Wehmeyer (4) directed attention to the influence of substrate on species of Diaporthe. He considered the effect of substratum on the character of the stromata and on the size and shape of the perithecial and conidial stages. He concludes that in some species stromatic characters are quite variable; in others, more or less constant; and that morphologic differences in perithecial stages and conidial stages are correlated with the genus or species of plant upon which the fungus is grown. He suggests furthermore that "species groups" may, in subsequent studies, be separated.

Apparently no species of Diaporthe, as has been stated, has been previously described on Delphinium. When, however, the larkspur pathogene is compared with the numerous forms described by Nitschke (2) it is found to correspond morphologically with *D. arctii* (Lasch) Nit.[1] This is a group species known to occur on stems of Lappa, Cirsium, Carduus, Tanacetum, and Centaurea. Nitschke states that a fungus on *Carduus acanthoides* which is labelled *Sphaeria orthoceras* = *Sphaeria achilleae* should be *D. arctii*. Auerswald described a *D. achilleae* on *Achillea millefolium* which he later identified as *D. orthoceras* Fr. The organism which Nitschke used as the basis of his *D. orthoceras* (Fr.) Nit. occurs on Achillea. However, Fries's *Sphaeria orthoceras* (Elenchus Fungorum II, p. 97), specimens of which were not available to Nitschke, occurs on Lapsana. If one emphasizes the importance of substrate these could all be distinct. If, on the other hand, one regards the slight differences to be the result of substrate, as indicated by Wehmeyer's studies (4) on Diaporthe, and as is most reasonable at the present state of our knowledge of this genus and other sphaeriaceous fungi, then they should all be regarded as one and the same. The writer therefore regards the larkspur pathogene as *D. arctii*.

SUMMARY

A brief account is given of a blight of larkspur caused by *Diaporthe arctii*. The pathogene possesses a Phomopsis stage, which appears on living parts, and an ascigerous stage, which develops on decaying stems. The genetic connection of these stages is established by cultures and by inoculation. Infections resulted from inoculation with ascospores and with conidia from culture from ascospores.

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[1] Thanks for this identification are due to Dr. C. L. Shear, U. S. Department of Agriculture, Washington, D. C., and to Dr. L. E. Wehmeyer, Ann Arbor, Mich., to whom specimens were submitted.

EFFECT OF SHADING ON THE RATE OF DEVELOPMENT OF TOMATO YELLOWS[1]

MICHAEL SHAPOVALOV AND J. W. LESLEY

It has been frequently assumed that the percentage of plants affected by curly top in sugar beets and by yellows in tomatoes depends simply on the degree of infestation with the beet leaf hopper (*Eutettix tenellus* Baker) which spreads these diseases. Variations in the geographical and seasonal distribution of tomato yellows have been explained as merely determined by variations in the prevalence of the hopper in different sections and seasons. The common observation that tomato yellows is less severe in orchards or in other shady places than in adjoining open fields has been understood to mean that the insect vector loves sunshine and abhors the shade. Since the beet leaf hopper is the only known carrier of the curly-top virus in the United States, unquestionably a correlation between the degree of infestation with the leaf hopper and the percentage of infection is to be expected. Nevertheless, some of the recent infection trials[2] have indicated very clearly that the development of the disease is also determined by the conditions to which the plant is exposed after inoculation, particularly by the intensity of light. When potted plants, all inoculated by the same method and otherwise treated in an identical manner, were placed under different light intensities, a greater proportion remained healthy when heavily shaded than when lightly shaded or not shaded at all. Our field trials at Shafter, California, in 1929, with plants exposed only to natural infection, present additional strong evidence of this. The progress of yellows in infected plants was delayed by shading, but the symptoms developed more rapidly after shading was discontinued.

The Stone variety of tomato and two closely related dwarf hybrid progenies were used in these trials. The plat consisted of two rows of unshaded Stone, two rows of unshaded dwarfs, and two rows of shaded dwarfs. It was subject to natural infection only. The shade was afforded by muslin tents extending over entire rows, fully open at the ends and with considerable openings on the sides, as shown in figure 1. Thus the intensity of light was reduced for the sheltered rows, although insect invasion of the tents was not entirely prevented.

[1] Joint contribution from the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C., and the University of California. Paper No. 227, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Shapovalov, Michael, and F. Sidney Beecher. Experiments on the control of tomato yellows. U. S. Dept. Agr. Tech. Bul. 189. 1930.

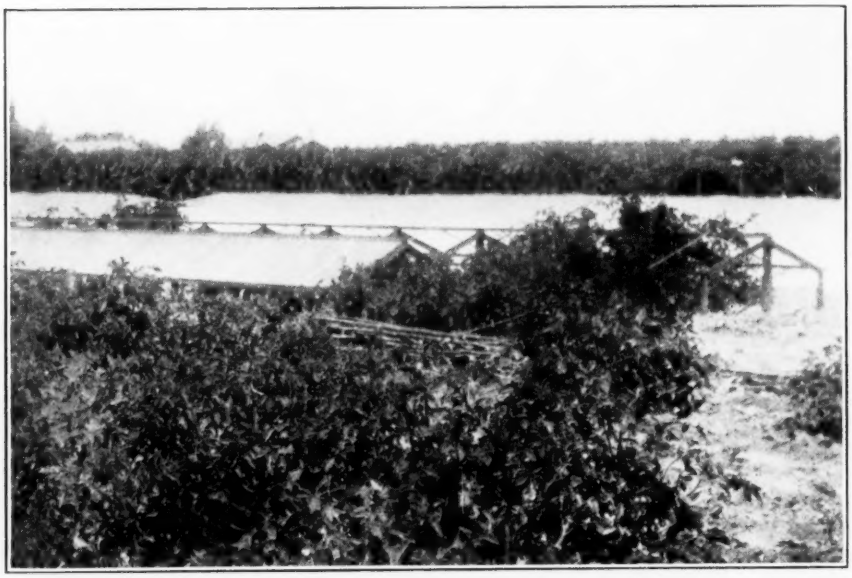

FIG. 1. The type of tents used at Shafter, Calif., in shading experiments with tomatoes.

As shown in table 1, unshaded rows of both varieties, the majority of the season's total number of cases of yellows had developed by June 11 and only a very small additional proportion by July 8 or later. In shaded rows no indication of yellows whatever was noticed until June 11 and subsequently for some days the development of the disease was slow. On July 5 the tents were removed. Three days later, on July 8, the number of affected plants in the two shaded rows had increased by 90 per cent and on July 26 by more than 100 per cent over that noted 11 days prior to the removal of the tents. In the unshaded rows, the rate of increase of yellows was much greater in the period June 11–24 (13 days) than in the period June 24–July 8 (14 days), whereas in the shaded rows the reverse occurred. These differences are shown in figure 2, where each line represents averages of the two rows of each kind.

These results indicate very clearly that many of the apparently healthy plants had become infected by invading leaf hoppers while these plants were still shaded by the tents. The environmental conditions under the tents were beneficial to the host plants and unfavorable to the virus. It is quite possible that the entire amount of infection in the shaded rows occurred on the same days as in the unshaded rows and that the delayed appearance of the symptoms of yellows under the tents signified merely a slower development of the disease. If the sudden increase of yellows after June 24 in the shaded rows was caused by new infection, there should have

TABLE 1.—*Progress of tomato yellows in shaded and unshaded rows at Shafter, Calif., in 1929*

Variety and treament	Total number of plants	Number and per cent of plants affected by yellows from the beginning of season up to dates shown below.													
		May 21		*May 31*		*June 11*		*June 17*		*June 24*		*July 8*		*July 26*		*August 2*	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Stone																	
Unshaded	51	1	2.0	30	58.8	38	74.5	38	74.5	41	80.4	41	80.4	43	84.3	43	84.3
Hybrid 006.18																	
Unshaded	51	1	2.0	15	29.4	27	52.9	31	60.8	32	62.7	32	62.7	35	68.6	35	68.6
"																	
Shaded	50	0	0.0	0	0.0	4	8.0	8	16.0	8	16.0	18	36.0	27	54.0	30	60.0
Stone																	
Unshaded	50	5	10.0	12	24.0	21	42.0	25	50.0	28	56.0	31	62.0	34	68.0	35	70.0
Hybrid 006.24																	
Unshaded	51	2	4.0	9	17.7	17	33.3	22	43.1	24	47.1	25	49.0	26	51.0	26	51.0
"																	
Shaded	49	0	0.0	0	0.0	5	10.1	5	10.1	11	22.4	18	36.7	20	40.8	23	47.0

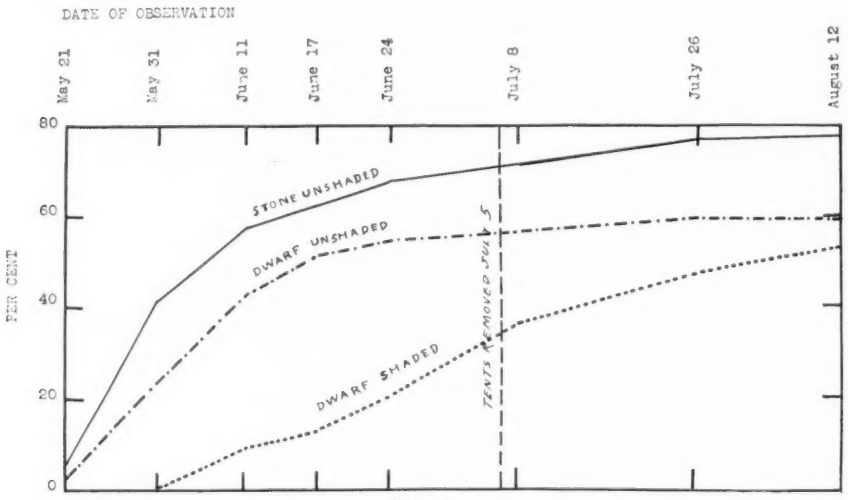

FIG. 2.

been a corresponding simultaneous increase in the unprotected rows, but such was not the case. Over 90 per cent of the season's total of yellows developed in the unshaded rows by the end of June. This is entirely in line with what might have been expected from previous experience under the conditions at Shafter. Certain counts taken there during the last week in June showed 98 per cent of yellows in 1926 and 95 per cent in 1927 and

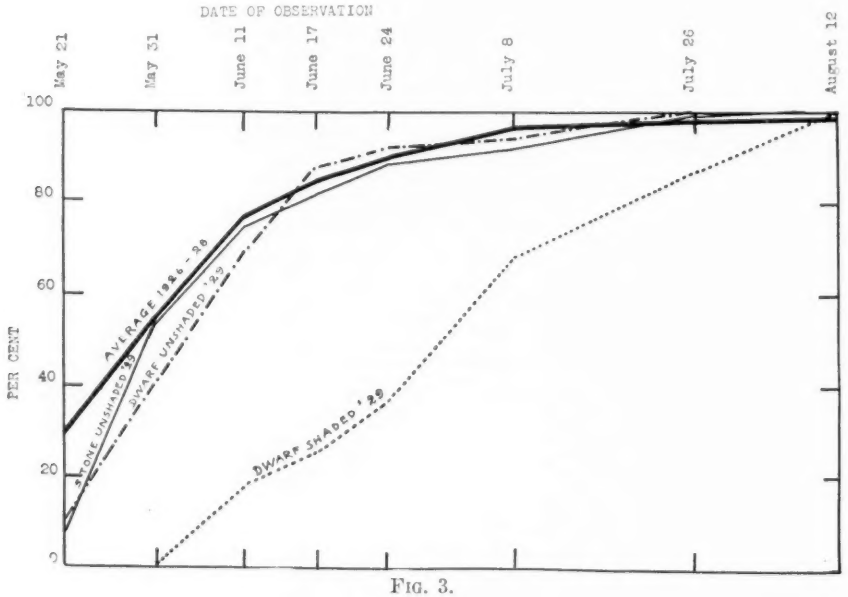

FIG. 3.

1928 in unshaded rows. The progress of the disease for these years is illustrated in figure 3. Furthermore, the period of 3 days between the removal of the tents and the next inspection on July 8 is far too short for the new infection to develop distinct symptoms of yellows in plants which had been growing for three months in the field. In previous inoculation field trials with viruliferous leaf hoppers (*E. tenellus*) only a very small percentage of very young plants (2 to 3 inches in height) showed early symptoms of yellows on the fourth day after the removal of the insects. Larger plants require a much longer incubation period. At Riverside, plants inoculated 3 weeks after transplanting required at least 16 days before the symptoms could be detected. Therefore, we may safely infer that most of the shaded plants became infected before the middle of June, as was apparently the case with the unshaded rows, and that the three days' exposure to the climatic conditions at Shafter in July following the removal of the tents, in addition to over two weeks of incubation under shelters, during which diffusion of the virus probably occurred, was sufficient to bring out the characteristic symptoms of yellows in those plants in which the virus was present.

It should be concluded from the foregoing data and discussion that proper shading of tomato plants in the regions characterized by the prevalence of the beet leaf hoppers only partially protects the plants from the invasion of the infecting hoppers and that it increases the tolerance of the plants to the virus, enabling them to produce a crop if the shading is continued after the infection takes place and may help some plants entirely to overcome it.

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NATURAL FIRE-BLIGHT INFECTIONS ON SPIRAEA VANHOUTTEI

A. B. Groves

The natural occurrence of fire blight on *Spiraea Vanhouttei* was first suspected by Rosen and Groves (1) after artificial infections were readily produced with pure-culture suspensions of *Bacillus amylovorus* (Burr.) Trev. The possibility of observing any natural infections then was rendered difficult by the prevalence of frost injury to the specimens available.

The most favorable conditions for the occurrence of natural fire-blight infections on *Spiraea Vanhouttei* existed at the Winchester Research Laboratory of the Virginia Agricultural Experiment Station, where there were two specimens located directly beneath a large pear tree. (Fig. 1). These were examined frequently to locate any infections that might occur under such favorable conditions. The first of what appeared to be fire blight on the Spiraea was observed on May 12, three days after the appearance of fire blight on the pear tree beneath which they were located. By this time the disease was general throughout the tree, particularly on the water sprouts which were abundant on the larger limbs. Several of the diseased twigs (Fig. 2) were removed and isolations attempted from them, together with one of the blighted pear twigs. Bacteria were obtained in abundance from all the material, the colonies being typical of *Bacillus amylovorus*. Transfers were made from these and suspensions in sterile distilled water were prepared from the transfers when 48 hours old. These suspensions were used in making inoculations into both young pear and Spiraea shoots which had been removed and placed with the cut ends in water in the laboratory. This precaution was taken to prevent possible external natural infection. The bacteria obtained from the blighted Spiraea produced typical blight on the pear and Spiraea shoots, as did the bacteria obtained from the pear. The first evidence of blighting from these infections was visible 40 hours after inoculation. The organism was readily recovered. The disease produced on the artifically infected Spiraea was very similar to that described by Rosen and Groves,[1] the shoot proper becoming flaccid and discolored, without apparent invasion of the leaves attached. Small droplets of ooze did appear, however. The disease advanced somewhat more vigorously on the inoculated shoots than on the naturally infected ones.

[1] Rosen, H. R., and A. B. Groves. Studies on fire blight: host range. Jour. Agr. Res. **37**: 493–505. 1928.

FIG. 1. Photograph showing the location of a *Spiraea Vanhouttei* plant directly beneath a pear tree. The numerous water sprouts may be observed, a number of which are blighted.

FIG. 2. Blighted shoots of *Spiraea Vanhouttei*, naturally infected.

A number of Spiraea plants are located in the planting about the building, but blight was found only on those directly beneath the blighting pear tree. The foregoing indicates that natural infections of fire blight do occur on *Spiraea Vanhouttei* under conditions favorable for such infections, as herein noted.

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CERCOSPORA BATATICOLA, N. SP., PARASITE OF THE SWEET POTATO IN AMERICA

R. Ciferri and S. C. Bruner

Cercospora batatae Zimmerman,[1] parasitic on the sweet potato, *Ipomoea batatas* L., was originally described from material of African origin and later reported from other countries of the oriental region, as has been noted in the work of Harter and Weimer.[2] A Cercospora is known also to occur on the sweet potato in America, and these authors call attention to the fact that the species found in Florida is possibly distinct from that of the Old World.

Through the courtesy of Dr. G. F. Weber, plant pathologist of the Agricultural Experiment Station of the University of Florida, we have obtained a specimen of the Cercospora of Florida which has been studied in conjunction with others from Cuba and Santo Domingo collected by the writers, and these, in turn, compared with a specimen from the Philippines obtained through exchange.

The results of a biometrical study of the conidia, measured by means of a filar micrometer and Bausch and Lomb 7.9 and 4 mm. objectives, are given in the following tables.

TABLE 1.—*Measurements of length of conidia of the American and Philippine species of Cercospora*

Class, microns	Florida	Cuba	Santo Domingo	Recapitulation 3 species	Philippines
41–50	1	2	0	3	1
51–60	2	4	2	8	2
61–70	1	6	2	9	9
71–80	3	9	3	15	14
81–90	7	4	12	23	6
91–100	10	4	7	21	2
101–110	6	2	4	12	1
111–120	2	2	3	7	0
121–130	1	1	2	4	0
131–140	0	1	0	1	0
141–150	1	0	0	1	0
151–160	1	0	0	1	0
Total	35	35	35	105	35

[1] Zimmerman, A. *Untersuchungen über tropische pflanzenkrankheiten,* Erste Mitteil. Ber. Land. u. Forstw. Deutsche Ostafrika 2: 11–36. 1904.

[2] Harter, L. L., and J. L. Weimer. *A monographic study of sweet-potato diseases and their control.* U. S. Dept. Agr. Tech. Bul. 99. 1929.

TABLE 2.—*Measurements of width of conidia of the American and Philippine species of Cercospora*

Class, microns	Florida	Cuba	Santo Domingo	Recapitulation	Philippines	Total
2	0	1	0	1	1	3
3	3	5	2	10	11	31
4	13	20	7	40	19	99
5	17	8	22	47	3	97
6	2	1	4	7	1	15

TABLE 3.—*Number of septae characterizing the conidia of the American and Philippine species of Cercospora*

Class, septae	Florida	Cuba	Santo Domingo	Recapitulation	Philippines	Total
2	0	0	0	0	1	1
3	0	0	0	0	4	4
4	0	1	0	1	8	10
5	1	1	0	2	10	14
6	2	2	1	5	6	16
7	1	4	1	6	3	15
8	3	8	2	13	2	28
9	4	9	2	15	0	30
10	7	6	10	23	1	67
11	5	2	10	17	0	34
12	7	1	5	13	0	26
13	1	1	2	4	0	8
14	2	0	1	3	0	6
15	1	0	1	2	0	4
16	1	0	0	1	0	2

The specimens recorded above were collected as follows:

1. Florida. Labeled: *Cercospora* sp. not *batatae*. On *Ipomoea batatas*. Homestead, Florida, 1, 23, 23. Coll. G. F. Weber.

2. Cuba. Labeled: *Cercospora* sp. on *Ipomoea batatas*, Wajay, Habana, 9, 26, 29. Coll. S. C. Bruner.

3. Santo Domingo. Labeled: *Cercospora* sp. On *Ipomoea batatas*. Colonia Jamao, Moca, 5, 14, 28. Coll. R. Ciferri.

4. Philippines. Labeled: *Cercospora batatae* Zimm. On *Ipomoea batatas* L., Los Baños, 16, 6, 19. Coll. ?

Although the Cercospora found on sweet potato in America shows considerable biometrical variation, yet, judging from the average measurements of the three sets of specimens studied, it appears to be distinct from the

Asiatic species in having longer and broader conidia and in being provided with a larger number of septae.

No difference was seen in the form of the conidia, which in all of the specimens is elliptic-elongate to vermiculate or subfiliform, being also similar in color, which varies from yellowish brown to light yellow. The conidiophores, furthermore, are almost identical; these vary from 80 to 140 μ in length by 2 to 4.5 in width and are provided with septae, being yellowish brown with their extremities paler.

As regards form, color, and size of the spots produced by *Cercospora batatae*, judging from the dried Philippine specimens studied, these are

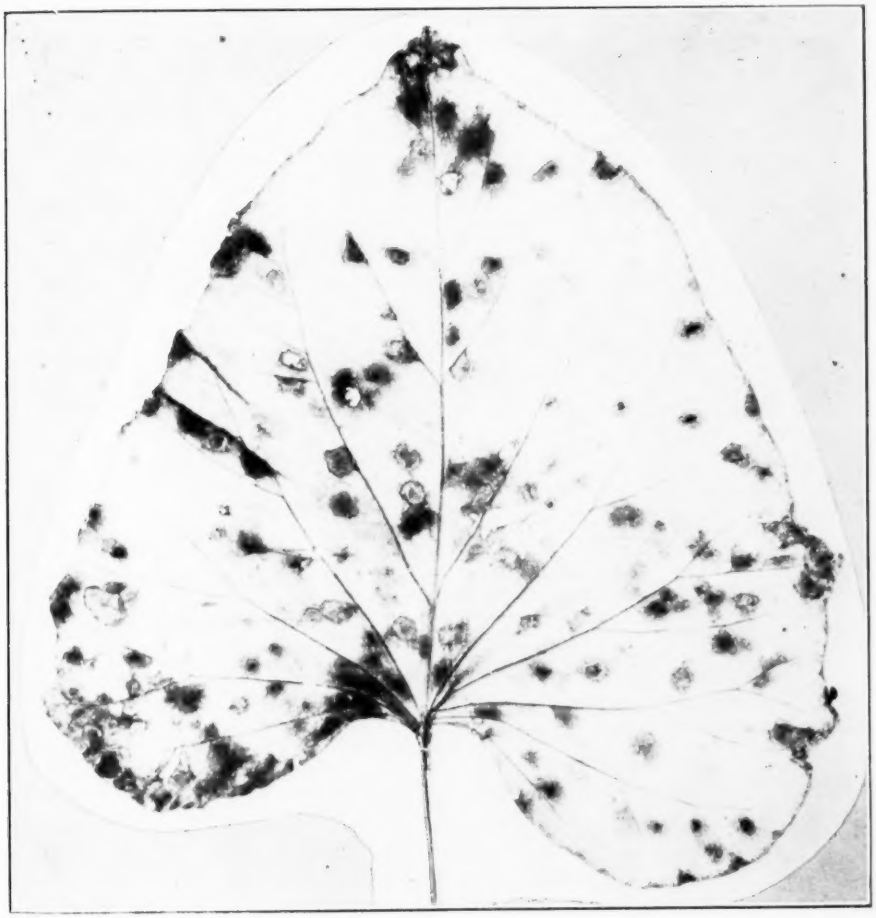

FIG. 1. Leaf of sweet potato attacked by *Cercospora bataticola*, n. sp. × ½. (Photograph by Dr. G. F. Weber.)

relatively large (usually 10 mm. or less in diameter, but sometimes as large as 15 mm.), dark, becoming almost black, irregular in shape but tending to be rounded, and usually confluent, rarely isolated. The specimens from Florida, Cuba, and Santo Domingo show smaller spots (normally 3–8 mm., rarely 2–10 mm.), isolated or subconfluent, purplish black in color, almost indefinite when young, and circular to irregular in outline (Fig. 1).

In view of these differences and in confirmation of the opinion expressed by Harter and Weimer,[3] we believe that the Cercospora found on sweet potato in America should be separated from that of the Old World as a distinct species, for which we hereby propose the name of *Cercospora batatícola*, n. sp. The reported occurrence of *C. batatae* in Brazil by Averna-Saccá[4] should be confirmed.

We are indebted to Dr. Weber for the accompanying photograph.

ESTACIÓN NACIONAL AGRONÓMICA,
MOCA, SANTO DOMINGO.
ESTACIÓN EXPERIMENTAL AGRONÓMICA,
HAVANA, CUBA.

[3] *Op. cit.*

[4] Averna-Saccá, R. *As molestias criptogamicas das plantas horticolas.* Bol. Agr. (São Paulo) **18**: 382–416, 486–515, 567–583, 634–654. 1917. Quot. by Harter and Weimer.

PHYTOPATHOLOGICAL NOTE

The relation of leaf blight to sun scald of honeydew melons.—During the summer of 1929 leaf blight, caused by *Macrosporium cucumerinum* Ell. and Ev., resulted in complete drying of foliage of many honeydew melons in the Arkansas River Valley of Colorado. In some instances every plant in a field was dried and prostrate on the soil, leaving the melons exposed to the sun. After the leaves had dried, spots of varying sizes began to appear on the fruit. This spotting was observed wherever the foliage had been destroyed. Melons in fields where there was very little leaf blight were not spotted. The spots were limited in every instance to the side exposed to the afternoon sun, thus indicating that solar radiation may have been the primary cause of the conditions (Fig. 1).

FIG. 1. Honeydew-melon field showing sun scald on the melons.

The first signs of sun scald are small brown spots upon the exposed side of the fruit. These spots enlarge slowly, as the season advances, until they attain considerable size. Spots varying from 1 inch to 5 inches in diameter were observed. The affected areas are usually round and become somewhat

sunken. They take on a dark color with age. A white to gray margin of chlorotic tissue usually surrounds the darker necrotic spots.

Not all the melons in a field were affected, the greener and less mature melons apparently being less susceptible than those that were nearly ripe. Cantaloupes and watermelons were likewise attacked with leaf blight, but these melons did not become spotted as did the honeydew melons.

Microscopic examination was made of freshly wounded tissue, but no bacteria or fungi were found. Numerous cultures were made on prune agar, potato-dextrose agar, and corn-meal agar; but no organisms developed. As the season advanced, however, and the spots became older, numerous secondary fungi and bacteria gained entrance and caused the fruit to rot. The affected fruits eventually became a soft, spongy, water-soaked mass. The fact that this condition occurred only where foliage had been destroyed and was not found where plants were unaffected with leaf blight indicates that the spots are due to sun scald. This possibility is further substantiated by the fact that the traumatic tissue is bacteriologically sterile at the onset of necrosis. It is known that ultraviolet light is present in large amounts at this elevation (3500 to 4000 feet) in the afternoon, and it is very probable that these rays are primarily responsible for the injury.

From the nature of the association of sun scald only with destruction of foliage by *Macrosporium cucumerinum*, it would seem logical to assume that any measures which would check the ravages of the leaf blight would also tend to prevent the sun scald of melons.—E. L. LEC'LERG, *Colorado Agricultural Experiment Station, Fort Collins, Colorado.*

BOOK REVIEW

Belling, John. The Use of the Microscope. 315 pp. McGraw-Hill Book Company, Inc.: New York, N. Y., 1930. Price $4.00.

This treatise on the microscope has appeared at a very opportune time. The attempt to keep pace with other branches of biological research has resulted in a marked increase in microscopical research and has brought about a need for just such a book as this, that shows how a microscope may be brought to its maximum efficiency.

The opening chapter gives 62 causes of injury to the microscopical image. Such an impressive list is enough to discourage all but the most optimtisic microscopist; however, in later chapters the author shows how both major and minor errors may be detected and corrected.

The following paragraphs on the light, the condenser, the object, the objectives, and the oculars show the author's method of adjusting for some of the major corrections of the high-power microscope.

He eliminates the glare from an incandescent lamp by a ground-glass filter and a diaphragm near the lamp to control the size of the light source. The intensity of the light is reduced by a light-absorbing combination of color filters. Minor reflections from the microscope mirror are eliminated by replacing it with a reflection prism.

He recommends the use of a condenser corrected for spherical and chromatic aberration; the latter correction may be neglected if use is made of the proper light filters. The condenser must be accurately centered and with these precautions the 16 mm. objective may be used with an almost full cone of light without objectionable glare.

The twin objective binocular microscope is well adapted for magnifications ranging from 10 to 100. The monobjective binocular microscope is best suited for long periods of study of objects requiring high magnifications. The object for such study is usually mounted on a glass slide and may or may not require a cover glass. All slides should be 1 mm. thick and the cover glasses should be 0.17 mm. thick to eliminate the necessity of constantly changing the correction collar of the objective or the tube length of the microscope.

The character of the mounting medium determines the proper type of objective to be used to obtain the maximum resolution and definition. The efficiency of the microscope centers around the objectives. They should be optically clean and accurately centered in the light axis. For low-power studies the 16 and 8 mm. apochromatic objectives are well adapted. The useful magnification of an objective ranges from 530 to 1060 times the working aperture and it is advisable to keep within this range. Water- and

oil-immersion lenses, depending on the character of the preparation, are often to be preferred to the high-power dry lenses.

The author considers the draw tube almost a nonessential feature of the microscope if slides and cover glasses of the proper thickness are used and the higher dry and water-immersion lenses are equipped with correction collars. All binocular microscopes, however, should have some way of preventing a change in tube length that occurs in most makes when the interocular distance is changed. It is infrequently necessary to make corrections by means of the draw tube when objects at different depths in the mounting medium are being studied.

Oculars of the compensating type, when light corrections are made by filters for achromatic objectives, are recommended by the author for general use, except in photography, where special oculars giving a flat field are essential. Oculars with a low magnification give a curved field and should be avoided by changing to a lower objective.

The author has used with great success water-immersion lenses. They are superior for material in a water medium if the object is more than 10 microns below the cover glass. They are, however, primarily for the skilled microscopist, owing to the accurate corrections that must be made with the collar if maximum resolution and definition are to be obtained. The reviewer does not see why microscopists need to make a hasty change from the highly perfected oil-immersion lenses to water immersions so much used by the author even for aceto-carmine preparations. It is customary to use only a thin layer of aceto-carmine and the pollen mother cells are normally in contact with both the cover glass and slide. With objects so prepared the major reason for using a water-immersion lens that requires accurate adjustment for each preparation is obviated.

It seems that many of the author's suggestions would have been more convincing if the optical reasons for the changes had been given. Perhaps the author has spared us by omitting complicated formulae which underlie his suggestions for improvement; however, the reviewer hopes that some capable critic will show that the author's suggestions are based on accepted optical theory.

Testing the microscope and adjusting it to its maximum efficiency have been made possible if the microscopist follows the instructions outlined in the chapters on tests and rules for high-power and routine microscopy. The star test detects axial spherical aberration, inaccurate tube length, and improper adjustment of the correction collar. With everything correct the disc or ring into which the small spot of light expands, when out of focus of the microscope, will be equally sharp at equal distances within and without the focus, though it may not be equally bright. The ring test is used

to correct the condenser. If properly focused the light of the aperture will be greatest and solid. Raising or lowering the condenser, only, reduces the aperture without giving marginal rings of light.

Doctor Belling has prepared an excellent glossary and index. The text is full of suggestions to help the student of the microscope acquire efficiency in its manipulation and at the same time reduce the fatigue that often comes from constant looking through hand lenses, twin objective binoculars, and high-power microscopes. There are many departures from the usual practice of slide preparation, adjustment of light and lenses, and making camera-lucida drawings and photomicrographs, which years of experience have shown to be practical.

Instructors and professors of biology in many of our universities will go through the next few semesters with a worried look. Their students will remind them that one of our leading microscopists tips his microscope at an angle of 45°. The use of a microscope stage tipped at an angle of 45° might prove very unsatisfactory for studying material mounted in an aqueous solution with immersion lenses if it allows the objects and the immersion liquid to settle to the lower side of the slide. Two of the leading microscope manufacturers, however, are producing microscopes with a horizontal stage and oculars tipped at an angle of 45°, thus eliminating the objectionable feature of tipping the microscope stage and the strain from bending over a microscope in the vertical position.

The general use of aceto-carmine is due to Doctor Belling's demonstration of its applicability as a chromosome stain. Each individual user likely has found some modification to make this killing and staining solution more applicable to his material. So it seems with these usfeul hints on microscope manipulation, microscopists will begin at once to eliminate some or many of their errors, but each worker is likely to find some modification of the rules to suit his particular need.—A. E. LONGLEY, *Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.*

ABSTRACTS OF PAPERS PRESENTED AT THE TWENTY-SECOND ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, CLEVELAND, OHIO, DECEMBER 30, 1930, TO JANUARY 1, 1931, INCLUSIVE

Effect of solid carbon dioxide upon transportation diseases. CHARLES BROOKS.

Solid carbon dioxide now furnishes a convenient means of increasing the carbon-dioxide content of storage air and has been used experimentally for this purpose in small containers, in pony refrigerators, and in standard refrigerator cars. Within a half-hour to an hour after a car is loaded it is possible to increase the carbon-dioxide content of the atmosphere to a percentage that will have a checking effect on the rotting and softening of warm fruit equivalent to a 30 to 40 degree F. drop in temperature. If the gas escapes within the next 18 to 24 hours no objectionable flavor is likely to result, but with peaches, strawberries, and red raspberries there is the possibility of a reduction in flavor and with more extreme treatments the fruit may become quite flat and insipid. Dewberries, blackberries, plums, and cherries are more resistant to carbon-dioxide injury. Grapes, sweet corn, peas, and beans seem to offer the greatest promise of beneficial effects without harm to the product. The inhibiting action of the gas ceases soon after restoration of normal atmospheres, but by this time the car has been fairly well cooled.

Further studies with psyllid yellows of the potato. B. L. RICHARDS.

In repeated tests the adult form of *Paratrioza cockerelli* in numbers up to 1,000 per plant has failed to produce symptoms of psyllid yellows on the potato plant. Nymphs from adults used in such tests, as well as from all other sources employed, when used in sufficient numbers, produced the disease uniformly. All attempts to date to separate nymphs from the infective principle by growing young nymphs on healthy plants from eggs hatched on healthy leaves in petri dishes have failed. The type of symptoms and the degree of injury to the potato appear to be definitely correlated with number of nymphs feeding, length of feeding period, and the intensity and duration of light exposure. Under greenhouse conditions psyllid yellows is not induced uniformly with fewer than 15 nymphs. With larger numbers, symptoms appear in from 4 to 6 days. The progress of the disease is interrupted, and the plant apparently may assume a normal expression, if the feeding nymphs are removed from the infested plant in 5 to 10 days after appearance of the first symptoms. Growth, which is stimulated by insect feeding, but which occurs subsequent to their removal, is to all appearances normal. In Utah, normal plants are obtained from tubers grown on psyllid-infested plants.

Aphids as vectors of leaf roll among sprouting potato tubers. F. C. STEWART and H. GLASGOW.

In June, 1928, and again in March, 1930, potatoes purchased at grocery stores in Geneva, N. Y., were found infested by large numbers of the spinach aphid, *Myzus persicae*. The occurrence of aphids on sprouting potato tubers is frequent in England and Ireland, but has not been previously reported from America. Experiments conducted by the writers show that the aphids may spread leaf roll among sprouting tubers.

In one experiment halves of 38 tubers from healthy plants were mixed with aphid-infested leaf-roll tubers and allowed to sprout for 20 days. The aphids multiplied and

spread to the sprouts of the healthy tubers. Each tuber-half was then cut crosswise into two pieces and all planted in pots in a greenhouse. Of the 76 plants produced 5 appeared normal, 68 showed positive symptoms of leaf roll, and 3 were doubtful. Under parallel conditions the other halves of the same tubers used as a check and not exposed to aphids produced 76 normal plants.

In another experiment it was proved that normal and leaf-roll plants may be obtained from different sprouts of the same tuber by allowing the aphids to feed upon some sprouts and excluding them from others.

The distribution of the latent virus in tubers of commercial potatoes. GROVER BURNETT and LEON K. JONES.

Tomato plants were inoculated with macerated leaf tissue of plants grown from commercial potatoes combined with macerated leaf tissue of tobacco plants affected with common tobacco mosaic. Apparently healthy plants from 93 commercial tubers were used in the tests with the following varieties represented: Netted Gem, Early Rose, White Rose, Wisconsin Pride, Burbank, and Beauty of Hebron.

One tuber of the Early Rose variety was found to be free from the latent virus. During the growing season in the field the foliage of four plants produced from this tuber remained free from the latent virus, while foliage of another plant from this tuber, after being inoculated with macerated tissue of a potato affected with rugose mosaic, produced streak in tomato when leaf tissue from it was inoculated in combination with tobacco mosaic.

The use of disinfectants in fertilizers for the control of potato scab and rhizoctonia. WM. H. MARTIN.

During the past 5 years a series of field and greenhouse experiments have been conducted to determine the value of the addition of various disinfectants to fertilizers for the control of soil-borne scab and rhizoctonia of the potato. The materials used included calomel, yellow oxide of mercury, formaldehyde, and various organic mercury compounds. These materials were mixed with the fertilizer and the resulting mixture was then applied in the open furrow in the usual manner, before planting the crop. Except where they were improperly incorporated in the soil, the potatoes developed normally and an appreciable reduction in scab and rhizoctonia followed. In an experiment in 1930 the following results were secured:

Treatment	Yield per acre	Free from scab	Severe rhizoctonia
	Bushels	Per cent	Per cent
Fertilizer alone	143.3	65.8	51.7
Fertilizer and 20 lbs. calomel per acre	176.0	93.9	2.7
Fertilizer and 10 lbs. yellow oxide of mercury per acre	147.9	97.4	3.1
Fertilizer and 45 lbs. Semesan per acre	170.7	93.7	1.8

Similar results have been secured in other experiments with potatoes. The scope of this work has been broadened to determine the value of this treatment in the control of soil-borne pathogenes attacking certain vegetables and ornamentals.

The effect of depth of planting and soil moisture on the development of rhizoctonia on the potato. E. S. CLARK and WM. H. MARTIN.

Cankering of the stems and stolons of the potato was much more severe in soils adjusted to 20 per cent moisture than in those with higher moisture content. The number of sclerotial bodies was likewise greater in the low- than in the high-moisture series. The disease was more prevalent on sprouts from seed pieces planted 3½ inches deep than on the sprouts of those planted 1½ inches. The severity of the disease at different planting depths was also influenced by soil-moisture content. Sprouts from seed pieces planted 1½ inches deep in a soil maintained at 20 per cent moisture showed more serious infection than those planted 3½ inches deep in a soil adjusted to a moisture content of 60 per cent. In general, however, deeper planting influenced the development of rhizoctonia in the same manner as a deficiency in soil moisture.

The effect of different pressures and of different types of lime in potato spraying. PAUL E. TILFORD.

In 1929 the increases due to spraying potatoes with 4–6–50 Bordeaux mixture were significantly greater when the spray was applied at 400 pounds pressure than at 200 or 600 pounds. In 1930, when there was very little rainfall to remove the spray, Bordeaux applied at 200, 400, and 600 pounds pressure gave practically the same results.

Experiments over a period of 4 years, comparing the increases obtained from spraying with Bordeaux prepared from stone lime, and from high calcium-hydrated lime, show hydrated lime to be as satisfactory as stone lime. Tests conducted by growers in commercial fields gave as good results from hydrated lime as from stone lime.

Bordeaux, prepared from high calcium-hydrated lime, gave greater increases than that prepared from high magnesium-hydrated lime.

The benefits from spraying in these experiments have been largely due to control of hopper burn and protection from the heat.

Seed treatment for damping-off of tomatoes. JAMES G. HORSFALL.

A chemical seed treatment of tomatoes for damping-off in seedling flats has been evolved for growers who have found soil steaming undesirable. Copper fungicides, notably 5 per cent $CuSO_4$ as a soak for 1 hour or as monohydrated dust and $CuCO_3$ dust, in 4 duplicate trials with naturally contaminated soil, increased emergence 205.3, 228.4, and 135.7 per cent and reduced disease in emerged seedlings to 20.0, 30.4, and 63.4 per cent of the check, respectively. In two tests the following additional copper compounds as dusts increased stands and decreased disease; cupric oxalate, tartrate, sulphide, silicate, arsenate, oxide, chloride, cuprous chloride, bromide, sulphide, thiocyanate, copper oxychloride, and malachite. Except for a slight lag, tomato seeds grew normally after soaking in an 8.6 per cent $CuSO_4$ solution for 24 hours or in a 50 per cent for 2 hours. Mercury compounds gave good disease control but injured germination under certain ill-defined conditions. Semesan, for example, reduced disease to 15.5 per cent of the check in 10 duplicate trials, but reduced emergence in one case to 7 per cent or incresed it in another to 171 per cent of the check. The average emergence, however, was 112.6 per cent of the check.

Effect of seed treatments on seed longevity. E. E. CLAYTON.

Seeds of many vegetable crops are often stored for 2 to 5 years before sowing. The use of recommended methods of seed treatment is greatly restricted by fear of seed injury. Results from six years' experiments with many kinds of seed show that this fear

is well founded. Recommended treatments with (1) mercuric chloride, (2) liquid organic mercurials, and (3) hot water have greatly shortened the life of the seed, even when they caused no immediately apparent injury. The dust treatments usually had no injurious after effects. Experimental work shows that much can be done to reduce or eliminate the harmful after effects of seed treatment. For example, with three lots of cabbage seed the germination 18 months after treating was:—

25 minutes at 50 degrees C. in water	51.60%
" " " " " " " .25% $ZnSO_4$	62.21%
Nontreated	53.30%

Other promising possibilities are indicated. The effective use of seed treatment requires close cooperation between seed producers, seed dealers, and the crop growers. An instance in which such cooperation has been successfully worked out with cauliflower seed is briefly discussed.

The growth rate of tomato plants affected by yellows. MICHAEL SHAPOVALOV.

The rate of elongation of tomato stems of both inoculated and uninoculated plants was ascertained by measurements made at different intervals. Three different types of curves, representing three different types of growth, were obtained. Healthy plants, whether inoculated or not, give practically the same, gradually rising curve (I). Inoculated plants which developed the disease and never recovered from it show at first a slight lag, then a sudden cessation of growth (II). Plants affected in the beginning, but later showing signs of recovery, are apt to lag at first, then show a rapid rise in the rate of growth, followed by a gradual increase, as in checks (III). Shading does not change the types of curves but reduces the number of positive cases and thus increases the number of plants giving (I) and (II) types of curves and speeds up the rate of elongation. In early stages of tomato yellows a slight decrease in the rate of growth of diseased as compared with healthy plants often precedes the appearance of other externally recognizable symptoms. The lag and the cessation of growth in these cases are accompanied by a rapid accumulation of carbohydrates and an increase in total nitrogen and, therefore, do not signify the weakening of either the photosynthesis or the absorption of nutrient elements from the soil. All these phenomena, however, suggest a serious disruption of the metabolic processes.

The occurrence of the Australian spotted wilt of tomatoes in Wisconsin. S. P. DOOLITTLE and C. B. SUMNER.

There appeared in the field at Madison, Wis., during the summer of 1930 a virus disease of tomatoes which seems identical with the so-called spotted wilt of tomatoes in Australia. The disease is a form of streak and the affected plants showed symptoms apparently identical with those described from Australia in a recent paper by Samuel, Bald, and Pittman. The young leaves developed the peculiar bronze markings typical of the Australian disease, the petioles and stem also being affected to an extent which occasionally resulted in the death of young plants. Fruits also were found showing the peculiar concentric markings figured in the Australian paper. The disease appeared on occasional plants during the season but did not spread rapidly in the field. It is readily transmitted to tomato by artificial inoculation but no work has yet been done on insect transmission. Its relationship to other forms of streak has not been determined.

Hybridization between Puccinia graminis tritici and Puccinia graminis secalis. MARGARET NEWTON, T. JOHNSON, and A. M. BROWN.

Crosses between forms 30 and 95 of *Puccinia graminis tritici* and a field culture of *Puccinia graminis secalis* have resulted in the production of four hybrid rust forms,

three of which have not hitherto been described. These hybrids, although distinguishable, resemble each other rather closely in pathogenic characters. They are much less virulent on wheat varieties than other known physiologic forms of the *tritici* series and are likewise less virulent on rye varieties than forms of the *secalis* series. If these hybrids are characteristic of all hybrids between the *secalis* and *tritici* varieties of *Puccinia graminis*, it is probable that such hybridization is not important as a source of virulent strains of wheat stem rust or rye stem rust.

A synthetic production of Puccinia graminis hordei E. and J.—MOSES N. LEVINE and RALPH U. COTTER.

By crossing pycnia of *P. graminis secalis* and *P. graminis tritici* on barberry, the writers recently obtained a strain of stem rust that attacks certain varieties of rye and wheat rather severely, which neither of the parents alone can do. Barley also is quite susceptible to this rust hybrid. These results indicate the production through hybridization of a rust form, not only pathogenically different from either one of the parents but possessing to a certain extent the parasitic properties of both. The actual existence of *P. graminis hordei* may here be involved. (Cooperative investigations between the Offices of Cereal Crops and Diseases and Barberry Eradication, Bureau of Plant Industry, United States Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Nuclear association in the aecium of Puccinia graminis. W. F. HANNA.

The sporidia of *Puccinia graminis* are uninucleate. Under certain conditions they have been found to produce secondary sporidia. Monosporidial infections on the barberry give rise, on the upper surface of the leaf, to pycnia containing many uninucleate pycniospores and, near the lower surface, to crescent-shape wefts of uninucleate hyphae. The stimulus of gravity does not appear to determine the particular area of the leaf in which these organs develop. About forty-eight hours after pycniospore-containing nectar from a pycnium of one sex is applied to a pycnium of opposite sex, binucleate cells appear in the hyphal wefts near the lower surface of the leaf. Shortly afterwards, multinucleate cells are found near the center of the weft; as many as sixteen nuclei have been counted in a single cell. Some of these binucleate and multinucleate cells arise by cell fusions. The paired nuclei of the aeciospores are considered to be descendants of nuclei which become associated in this manner. In one preparation a nucleus was found which appeared to be "migrating" through the cell wall into a neighboring cell. A few germinating pycniospores have been found, but their rôle in initiating aecial development is not fully understood.

Indications of heterothallism in Tilletia tritici. H. H. FLOR.

In a series of greenhouse and field tests wheat seedlings were inoculated with one or more cultures from single primary sporidia of *Tilletia tritici*. Ten such monosporidial cultures inoculated singly into wheat seedlings produced no infection. Of six trials in which the seedlings were inoculated with a mixture of two such monosporidial cultures, infection was obtained in three. Three trials in which ten of the monosporidial cultures were mixed produced infection in every case. In no instance were uninoculated plants infected. (Cooperative investigations by the Washington Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.)

Physiologic forms of bunt of wheat and varietal resistance. E. N. BRESSMAN.

Studies were made over a three-year period of the parasitic behavior of 100 collections of bunt, *Tilletia tritici* and *T. levis*, obtained from widely separated sections of the United States and several foreign countries. Ten standard varieties, nine reported as resistant and one as susceptible to bunt, were used as differential hosts and subjected to the usual inoculation tests. The results indicated that there are at least 10 physiologic forms of bunt, six *T. levis* and four *T. tritici*. Practically all of the varieties of wheat, formerly classed as resistant to this disease, are susceptible to one or more of these forms. White Odessa, Martin, Banner Berkeley, Albit, Regal, Sherman, Stephentshka, Cooperatorka, Hope, and Hussar are susceptible to several forms. Ridit, although resistant to most forms and collections, is susceptible to some forms. Turkey × Bd. Minn. No. 48, Hohenheimer "behaart" and Hohenheimer "unbehaart" are rather resistant to most of the forms but some infection is obtained. Rye was susceptible to several of the collections. *T. secalis* is designated as a form of *T. tritici*. Hosar, a selection by the writer, from Woolman's hybrid, Hussar × Hohenheimer "behaart" has been consistently resistant to all of the available collections of bunt.

Germination of wheat stem-rust teliospores formed in the greenhouse. THORVALDUR JOHNSON.

Attempts were made by freezing and by alternate wetting and drying to shorten the dormancy period of teliospores formed in the greenhouse. Either of these treatments, in most cases, reduced the period of dormancy. A combination of the two, that is, a short period of freezing (2–7 days) followed by alternate wetting and drying, invariably resulted in germination. Teliospores formed at low temperatures, 55°–60° F., germinated more abundantly when subjected to this treatment than those formed at higher temperatures, 70°–75° F.

Teliospores formed in nature on *Hordeum jubatum* and *Agropyron repens* in September, 1929, likewise responded to freezing followed by alternate wetting and drying and germinated abundantly at the beginning of December. Teliospores on *Hordeum jubatum* and wheat collected out-of-doors, September 3, 1930, commenced germinating September 19, after being subjected to the same treatment.

As to the minimal dormancy period of teliospores produced in the greenhouse, the shortest period between the completion of teliospore formation and the germination of the spores was 20 days. Spores frequently germinated between 30 and 40 days after formation. The shortest period from the inoculation of wheat plants with urediniospores to the germination of the teliospores formed on them was 55 days.

Effect of mineral nutrition on the reaction of wheat varieties to leaf rust. K. D. DOAK.

Wheat varieties showing various types of reaction to one physiologic form of leaf rust, *Puccinia triticina*, were grown in sand cultures providing different degrees of excess and deficiency in nitrogen, phosphorus, and potassium. Nitrogen increased susceptibility, while phosphorus and potassium decreased it. Excess nitrogen induced the development of larger primary uredinia and more abundant secondary uredinia and decreased chlorosis. Phosphorus in excess increased chlorosis, did not reduce the size of primary uredinia, and retarded or prevented development of secondary uredinia. Excess potassium increased chlorosis and also decreased the size of primary uredinia. Secondary uredinia appeared in incomplete rings around the primary. In varieties of intermediate reaction, excess nitrogen increased the percentage of infected points with uredinia, while nitrogen deficiency, excess phosphorus, and excess potassium decreased it. Phos-

phorus deficiency decreased chlorosis in both susceptible and intermediate varieties, but the uredinia were always small. Potassium deficiency decreased chlorosis but did not reduce the size of uredinia. A few resistant varieties were affected like the intermediates. The relation of time of starvation and excess to change in reaction was tested in water cultures.

The effect of temperature and light on the development of the uredinial stage of Puccinia graminis. LEONARD W. MELANDER.

Urediniospores of *Puccinia graminis*, hardened ten days by exposure to 0 to 1° C., withstood low temperatures on dry glass slides in a freezing chamber better than non-hardened spores. Hardened urediniospores of wheat, timothy, and oat stem rust survived at −29° C. to −40° C. for 40 to 45 days. After six days of alternating high and low temperatures the percentage of viable, hardened spores was lowered.

At 10° C. uredinia appeared a week later than at 20° C. They developed slowly at 0 to 1° C., and sometimes the infection type differed from the normal type produced at 10° and 20° C. *P. graminis tritici* form 36 produced numerous uredinia after 59 days at 0 to 1° C.; form 35 produced minute uredinia on Little Club wheat; form 15 generally failed to produce uredinia within 80 days. When reexposed to 20° C., type-1 uredinia became type 3; and form 15, dormant at 0 to 1° C. in wheat plants, produced normal uredinia.

Production of telia on wheat, oats, and rye was stimulated at 0 to 1° C. Both rust and host withstood −10° C. for 24 hours.

Low light intensities retarded stem-rust development. Urediniospores of *P. graminis tritici* form 15 formed in a light intensity of 302 foot-candles at 20° C. were longer and better able to withstand temperatures of −29° C. to −40° C. for 24 hours than spores formed in higher or lower light intensities. (Cooperative investigations between the Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Relationship of the oat smuts. W. F. HANNA and W. POPP.

Experiments in which oat seedlings inoculated with cultures of *Ustilago avenae* and *U. levis* were grown to maturity in the greenhouse yielded the following results: (1) Plants inoculated with a single monosporidial culture of *U. avenae* or *U. levis* did not produce smutted heads. (2) Plants inoculated with two monosporidial cultures of opposite sex produced smutted heads. If the two cultures were of *U. avenae* the infected heads were "loose" in appearance and their spores echinulate; if of *U. levis* the heads were "covered" in appearance and their spores smooth; if one of the cultures was of *U. avenae* and the other of *U. levis* the infected heads were somewhat variable in appearance, but upon close examination they proved to be of the loose type, and their spores were echinulate. (3) The sporidia of *U. avenae*, like those of *U. levis*, are of two kinds, (+) and (−); the sporidia of one species mate without difficulty with sexually opposite sporidia of the other species.

These results indicate that *U. avenae* and *U. levis* are genetically distinct with respect to the characters by which they are differentiated, but the ease with which crosses can be made between them suggests that they are closely related species.

Host specialization and parasitism of the genus Rhynchosporium. RALPH M. CALDWELL.

Results of additional experiments have now been secured with monosporous cultures of the scald fungus, *Rhynchosporium secalis* (Oud.) Davis from *Agropyron repens*, *Bromus inermis*, *Dactylis glomerata*, *Elymus robustus*, barley, and rye collected in

different localities. Two to five isolations from each host have been tested. The hosts inoculated included rye, barley, oats, wheat, *Danthonia californica*, *Hordeum jubatum*, and *Lolium perenne*. No culture infected any host other than that from which it came. In these cases significant infection almost invariably occurred. There is evidence of five host-specialized races of *R. secalis*. Excepting the scald fungus on Dactylis, no significant morphological variations were found within the species *R. secalis* as it occurred upon several hosts. However, upon *Dactylis glomerata*, the fungus is clearly distinct in morphology and parasitism. It is being described as a new species. The two species develop on the hosts in a similar and unusual manner. Penetration is direct from appressoria of conidial germ tubes. The fungus body consists primarily of a subcuticular stroma with very limited and delayed growth of hyphae in the mesophyll.

The development of crown gall, hairy root, and callus under controlled conditions. A. J. RIKER, W. M. BANFIELD, and G. W. KEITT.

Crown gall, caused by *Phytomonas tumefaciens;* hairy root, caused by *Phytomonas rhizogenes;* and wound overgrowth, caused by girdling, were each produced under conditions designed to avoid the presence of complicating factors. This was done by inducing these enlargements (1) on nursery apple trees grown in steamed soil both in the field and in the greenhouse and (2) in the field on stems of nursery apple trees, the treated parts of which were enclosed in small chambers designed to permit a disinfectant treatment and to prevent foreign organisms from gaining entrance. Cultures of single-cell origin and tested pathogenicity were employed.

After one and two years the results showed that inoculations with the crown-gall organism induced only crown gall, those with the hairy-root organism induced only hairy root, and girdling induced only callus and wound overgrowth. Mixed cultures of the crown-gall and hairy-root organisms caused malformations which showed complete intergradations of crown-gall and hairy-root characters. The pathogenic organisms gained entrance into the host plants readily through wounds, but callus appeared not ordinarily to be an open infection court for either of these bacteria. (Cooperative investigations between the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station.)

The life cycle of the hairy-root organism on apple in relation to pathogenesis. E. M. HILDEBRAND.

The life cycle of *Phytomonas rhizogenes*, the hairy-root organism, is being studied in relation to pathogenesis on nursery apple trees.

The entrance of the bacteria into the host tissues is accomplished only through wounds which heal so rapidly that the bacteria are unable to enter a wound three days old. The callus tissue which develops following a wound was determined not to be commonly an open infection court. Different kinds of insects were observed to be feeding on the callus, roots, and diseased tissue below ground. Efforts to isolate crown-gall and hairy-root bacteria from these insects have been unsuccessful.

The exit of the bacteria from the overgrowths seems to be a continuous process from the surface, where the organisms commonly occur in abundance. As the enlargements become older they contain correspondingly more xylem elements, from which the bacteria are less easily isolated. These bacteria may overwinter in the soil and may exist there separated from the host for over a year. (Cooperative investigations between the Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Wisconsin Agricultural Experiment Station.)

Physiologic specialization in Gymnosporangium. DONALD E. BLISS.

Statements from various parts of the United States are much at variance regarding the relative susceptibility of certain varieties of apple, *Pyrus malus*, to *Gymnosporangium juniperi-virginianae* and *G. globosum*.

Collections of *G. juniperi-virginianae* from Iowa, Kansas, and Wisconsin, when placed on the Tolman and York Imperial varieties, caused only flecking. However, *G. juniperi-virginianae* from West Virginia produced aecidial cups on the same hosts. Bechtel's Double-flowering crab, *Pyrus ioensis*, was highly susceptible to all of these collections. Varieties of apple trees secured from nurseries in Iowa, Kansas, New York, and Virginia were alike in their reactions. These data are indicative of physiologic specialization in Gymnosporangium.

Further inoculations were made, using three collections of *G. globosum* from Iowa. All of these caused an abundance of aecidia to form on *Crataegus mollis*, but flecking was the only symptom to develop on the Fameuse, Tolman, Yellow Transparent, York Imperial, Wealthy, McIntosh, Baldwin, Delicious, and Northwestern Greening varieties. Although the aecidia of *G. globosum* were found commonly in Iowa on *Crataegus* spp., none were observed on the leaves of 150 varieties of apples examined in 1930. *G. globosum* does not seem to occur on apples grown in the nursery in Iowa, although it has been identified on many varieties in New York.

Pathogenicity of three red cedar rusts that occur on apple. P. R. MILLER.

Successful inoculation was obtained with *Gymnosporangium germinale*, quince rust, on the fruit of Delicious, Winesap, Stayman, and Wealthy apple varieties and on quince foliage; with *Gymnosporangium juniperi-virginianae*, apple rust, on the fruit and foliage of Rome, Ben Davis, Grimes, and Wealthy and on the foliage of Jonathan; and with *Gymnosporangium globosum*, hawthorn rust, on the foliage of Maiden Blush, Rome, Ben Davis, Jonathan, Grimes, and Wealthy, and in addition on pear and *Sorbus* sp. These results are more or less in accord with the natural infection as observed in 1929. Apple fruits were susceptible for approximately fifteen days after petal fall. Infection was obtained through wounds on apple leaves which had become resistant. Leaves removed from the tree at the end of the day remained alive two months on a sucrose solution in Petri dishes. Indications of heterothallism were secured by mixing the pycnial exudates. Overwintering of aeciospores resulted in a marked increase in the percentage of germination. On an awn-leaf type of red cedar large galls of *Gymnosporangium juniperi-virginianae* were found on large limbs.

Brooks fruit spot. H. C. YOUNG and FRANK WINTER.

During the season of 1928 "fruit spot" caused a severe loss in the apple crop in the Ohio valley. Previous to this nominal losses occurred in widely separated orchards in southern Ohio but no general epidemic had occurred. Consequently, not much attention had been given to control measures.

A detailed study of the causal fungus, *Mycosphaerella pomi*, was begun in the spring of 1927. It was found that the perfect stage of the fungus occurs on almost all types of deciduous leaves throughout the State. The ascospores mature earlier in northern than in southern Ohio.

The humidity, temperature, and host relationships of the fungus are extremely interesting. The optimum temperature for growth is 22° to 24° C. Growth was almost stopped at 30° C.

It was found that dilute Bordeaux mixture (1–3–50) controls the fungus. During the season of 1929 the 4-weeks spray was the most effective. On account of the drouth no results were obtained in 1930.

Cultural characters and host range of the apple sooty-blotch fungus. M. W. GARDNER and R. C. BAINES.

By partial surface sterilization pure cultures of the sooty-blotch fungus, *Glocodes pomigena*, have been obtained from apple fruits. The fungus grows very slowly and on potato agar produces a thick black thallus on and around which gelatinous masses of spores accumulate. The fungus grows well on a variety of media but has sporulated only on potato, malt-extract, and prune agars. On very moist agar the spores bud profusely. Cultures similar to those from apple have been obtained from sooty blotch on the young wood of leatherwood, *Smilax hispida*, prickly ash, pawpaw, *Vitis cordifolia*, white ash, tulip tree, hard maple, willow, hawthorn, blackberry, sassafras, redbud, bladdernut, *Evonymus americana*, and *Cornus rugosa* and from fly speck on the first ten species above mentioned and sycamore, spice bush, and *Cornus alternifolia*. Sooty blotch has been produced on apples by inoculation with spore suspensions from cultures from sooty blotch on apple, pawpaw, and Crataegus and fly speck on white ash and sycamore. The incubation period in a cool moist compartment in the greenhouse was about three weeks and, in the orchard, much longer.

Artificial infection of fruit with the apple-scab fungus. C. O. BRATLEY.

The length of the period of susceptibility of apple fruit to infection by *Venturia inaequalis* is important because of its relation to the value of late-summer applications of fungicides and to the appearance of new lesions on the fruit in storage.

Inoculations during the summer of 1930 were made by rubbing conidia from infected leaves directly on the moistened surface of attached McIntosh fruit. The inoculated apples were immediately inclosed in small moist chambers made of wire netting covered with wet cotton, the whole then being inclosed in bags made of transparent moisture-proof cellophane.

When continuous wetting was maintained for 40 or more hours the inoculations were usually successful but when the fruits were dry at the end of 28 hours they failed. Inoculations made during August indicated that the length of the incubation period varied from one to two months, depending on the length of the moist period at inoculation. Infections resulting from inoculations made the last of August appeared six weeks later, three weeks after the apples had been picked and placed in storage.

Bagging experiments conducted on the same variety of apples showed that natural infection occurred as late as August 13.

The relation of root-feeding arthropods to crown-gall infection on raspberry. W. M. BANFIELD.

Seasonal-development studies of crown gall on red raspberry, grown in inoculated field soil during 1928 and 1929, show that (1) 92 per cent of plants became infected in the first season, averaging 5.8 infections per plant. (2) Infection appeared to occur at random on all underground parts and did not seem to be correlated with particular stages of development of these parts. (3) Infection was most abundant on roots and stems in the upper four inches of soil. (4) Infection commonly occurred through arthropod injuries.

No crown gall occurred on 118 plants during 1929 and 1930 in inoculated soil from which root-feeding arthropods were excluded except at points mechanically injured. Crown gall occurred on 63 of 76 check plants grown without arthropod exclusion, averaging 5.2 galls per diseased plant.

White grubs, *Phyllaphaga* spp., were found to be the dominant root feeding-form present in raspberry plots in August, 1930. The injuries found on plants in these plots,

through which infection commonly occurred, appeared to be due to grubs. In pot and cage experiments, using inoculated soil, grubs caused injury to raspberry roots identical to that found in the plots. Crown gall commonly developed at these grub injuries.

Crimp, a nematode disease of strawberries. A. N. BROOKS.

Crimp, also known as French bud, briar bud, etc., has been present in Florida for many years. It probably is identical with dwarf plant of Louisiana and red plant of England. Apparently, crimp is more destructive in Florida than elsewhere.

Crimp is fundamentally a bud disease affecting the development of young leaves. Affected leaves range in size from mere rudiments to almost normal, are crimped, darker green, and more brittle than normal, less pubescent, and usually reddish in places.

Nematodes, *Aphelenchus fragariae*, most numerous, were found abundant in diseased buds, *e.g.*, 7,597 nemas in 31 buds, and practically none in healthy buds, 2 nemas in 30 buds. These nemas were found to be ectoparasitic, not endoparasitic.

Uniformly successful inoculations were made by introducing suspensions of the nemas into buds of healthy plants. Hypodermic-needle inoculations with the filtered extract of diseased plants did not produce crimp in healthy plants. Strawberry aphids and red spider did not transmit the disease from crimped to healthy plants.

Warm water treatment of crimped plants, 48° C. for 20 minutes, killed the nemas in the buds and restored the plants to normal growth.

Control consists of setting clean plants in clean soil and roguing.

Anthracnose of strawberry caused by **Colletotrichum fragariae**, *nov. sp.* A. N. BROOKS.

Anthracnose, a spot disease occurring chiefly on strawberry runners, and occasionally on petioles, has been observed in central Florida for the past four years. The disease appears at its worst during the summer rainy season. The chief damage results from the girdling of runners before the young tip plants have put out roots and become self-supporting. Some fields have shown a 50–75 per cent kill of young plants. The average for the entire area is 2–5 per cent.

The mature diseased spots are from one to several centimeters long, girdling the runners, sunken, brown to black in color, the line of demarcation between the healthy and diseased tissue being quite sharp. Under a strong hand lens tufts of setae can be observed in profile in the spots.

The disease has been proved to be caused by a species of Colletotrichum, for which, since it appears different from other described species, the binomial **Colletotrichum fragariae**, nov. sp., is suggested.

Spraying with Bordeaux mixture 4–4–50 at ten-day intervals has been found greatly to reduce the amount of the disease but, because of frequent rains, does not completely control it.

Melons resistant to powdery mildew. IVAN C. JAGGER and G. W. SCOTT.

Powdery mildew (*Erysiphe cichoracearum* DeC. ?) first appeared in destructive form in the extensive melon, *Cucumis melo*, fields of Imperial Valley, California in 1925 and has been more or less injurious every season since, causing particularly heavy commercial losses in 1926 and 1930. Since 1926 a large number of melon varieties have been tested each season. During the first 2 years only limited resistance was found, but in 1928 mixed lots of seed from India gave numerous plants which were almost immune from mildew. These melons were commercially useless, as they had unpleasant flavors and poor shipping qualities. Crossing the resistant melons with the leading commercial

varieties of cantaloupes or muskmelons, followed by back crossing and repeated selecting, is beginning to give strains of melons which combine the commercial and eating qualities of the American varieties with the resistance of the plants from India. The long growing season in Imperial Valley makes it possible to grow two or three generations each season. In crosses there is a Mendelian segregation of the mildew-resistance character which is at least partially dominant in heterozygous plants. At best 2 or 3 years more will be required to purify resistant varieties of good commercial types.

Three new wilt-resistant varieties of watermelons. DUKE V. LAYTON and J. J. WILSON.

Three wilt-resistant varities, Pride of Muscatine (K–84), Iowa King (Q23), and Iowa Belle (Q21), grown on 30 acres of heavily *Fusarium niveum* infested soil, produced 27,000 pounds per acre of marketable melons in 1930. Twenty three acres of Pride of Muscatine yielded 3,111 pounds of seed and showed a wilt resistance, at blossoming time, of 70 per cent as compared with 20 per cent in the commercial checks of the var. Kleckley Sweet. Iowa King in a six-acre field yielded 804 pounds of seed and showed a wilt resistance of 60 versus 5 per cent in the commercial check. Iowa Belle, grown on one acre, produced 175 pounds of seed and gave a wilt-resistance response of 80 versus 23 per cent in the commercial check. Four thousand pounds of seed of the above-named wilt-resistant varieties is subject to distribution.

Iowa Belle showed less anthracnose (*Colletotrichum lagenarium*) injury in greenhouse trials and in the field than either Pride of Muscatine or Iowa King when exposed to infection.

Anasa wilt of cucurbits. L. RAY ROBINSON and B. L. RICHARDS.

Squash and pumpkin growing has been completely abandoned in many parts of Utah owing to a peculiar wilt, resembling in many respects the cucurbit wilt induced by *Bacillus tracheiphilus*. Recent experiments at the Utah station indicate that the wilt is not parasitic in nature but that the squash bug, *Anasa tritis*, is alone responsible for the disease. Wilting results in from 24 hours to 16 days, dependent upon the age of the plant, the progress of the season, and the number of insects feeding. When wilting is not too complete plants uniformly recover upon the removal of insects, and subsequent terminal and axillary growth is always free from disease symptoms.

Wilting results only above the point of insect contact whether the petiole or the entire stem is exposed to feeding. The rate of wilting and the few insects required to produce complete wilting suggest the possibility that a toxic substance injected by the insect during the feeding process is involved. All varieties of squash and pumpkins, together with the watermelon, cantaloupe, and cucumber, appear equally susceptible.

The writers suggest the name Anasa wilt of cucurbits.

Commelina nudiflora, a monocotyledonous host of celery mosaic. S. P. DOOLITTLE.

A mosaic disease, recently the cause of serious losses in Florida, shows characteristics differing from those previously reported on celery, although it is not certain that a different virus is concerned. The unusual feature of the disease is its frequent occurrence on a monocotyledonous host, *Commelina nudiflora*, from which it is transmitted both to celery and cucumber by *Aphis gossypii*, the host in question being a major source of primary infection to celery in the field.

The disease is transmissible by artificial inoculation and by *A. gossypii*, which is an important agent in its dissemination. It is transmissible to cucumber, tomato, tobacco, pepper and ground cherry, the symptoms resembling those of cucumber mosaic, with which the celery virus is probably identical.

The early symptoms are a pronounced leaf mottling and a downward, spreading curvature of the leaf stalks, some of which develop extensive brown, sunken streaks which are especially characteristic of the disease. The later growth of such plants shows little evidence of the disease other than a mild mottling of the leaves and a slight dwarfing. The browning of the leaf stalks, however, may reappear at intervals and often renders the plant unmarketable.

Two Septorias as a cause of late blight on celery. L. C. COCHRAN.

Two distinct species of Septoria have been shown to be responsible for distinct types of the late blight of celery. One causes a large spot, definite in outline, and, under conditions of proper humidity, small scattered pycndia develop in the central portion of the lesion. This type has been identified with the specimens of Briosi and Cavara and with the specimens of Chester (Annual Rept. Delaware Agr. Exp. Sta. 4: 63–65. 1891), which he named *S. apii* (Br. et Cav.) Chester. The other species produces a small spot, indefinite in outline, which is surrounded and densely crowded with black pycnidia. This type has been identified with Dorogin's species which he found in Russia and named *S. apii graveolentis* (Materialye po Mikologia i Fitopatologii Rossii 1: 57–76. 1915.)

Both of these forms have been found throughout the United States, but the type producing the smaller spot (*S. apii graveolentis*) is more common and more destructive. From experiments which are still in progress, both Septorias have been found to be serious parasites of celery leaves but only the small-spot type has been found to infect the leaf stalks.

Comparative histology of three bacterial blights of beans in the seedling stage. W. J. ZAUMEYER.

The relation of *Bacterium phaseoli* and *Bact. medicaginis* var. *phaseolicola* to the tissues of seedling beans is very similar and the organisms are difficult to distinguish except by cultural means. Being Gram-positive and invading the xylem cells to a greater extent than those of the parenchyma, *Bact. flaccumfaciens* is differentiated from the other two, which are Gram-negative and invade the parenchymatous tissues more than the xylem cells.

Bacterium phaseoli and *Bact. medicaginis* var. *phaseolicola* often produce large bacterial cavities in the parenchymatous stem tissues in close proximity to the cotyledonary node, whence they may migrate to the surrounding tissue by way of the intercellular spaces. They may also enter the xylem cells, traveling mostly in an upward direction and may pass through the entire plant, often causing death of the growing tip. Passage of the organisms from cell to cell, either by dissolution of the wall or internal pressure in the vascular tissue, is common.

Similar to the other two organisms, *Bact. flaccumfaciens* may break out from the vascular tissue, causing lysigenous cavities contiguous to the vascular strands from which they came, but the organism does not readily migrate into the surrounding parenchymatous tissues.

A wilt of beans caused by Pythium. L. L. HARTER and W. J. ZAUMEYER.

A wilt of beans caused by *Pythium butleri* was observed and the organism isolated coincidentally at Arlington, Virginia, and Greeley, Colorado, on the Late Stringless Green Refugee variety during the summer of 1930. The disease was first observed about the middle of July on plants from 8 to 16 inches in height. Species of Pythium have been isolated from beans before, but the disease has never been observed under abnormally high temperatures, such as prevailed during the past summer.

The earliest symptoms were observed at the soil level. The decay did not extend much below this point but rather extended upward into the lower branches of the plant. The cortex readily separated from the vascular tissues. The rot was particularly noted at the pulvini of the petioles and leaflets.

A slight flagging of the leaves was first noted in the day time, the plants partially regaining their turgidity at night. A day or two later a distinct wilting occurred, the plants dying soon thereafter.

Refugee beans were successfully infected in the greenhouse and the organism reisolated. The checks remained healthy. Wilting of inoculated plants did not take place at ordinary greenhouse temperatures, but, when they were placed in an infection chamber at a temperature of about 30°C., it occurred in 3 days.

Nature of powdery-mildew injury to snap beans in Virginia in 1930. H. T. COOK.

Powdery mildew caused a loss of over 50 per cent in the fall crop of snap beans in Virginia in 1930. Although a fair yield was obtained, considering the dry season, the beans were unmarketable after the first picking due to a russeting and spotting of the pods by the powdery-mildew fungus. Observations indicate that the disease was less severe on the Refugee than on the Bountiful and Wax varieties.

Correlative studies on the bacteriology of bean mosaic and seed transmission of the virus. RAY NELSON.

Isolations from cage- and field-grown bean plants in 1930 gave the following results: from apparently healthy plants—29 negative cultures from cage-grown Great Northern; 39 negative cultures from cage grown Robust; 28 negative cultures from field-grown Refugee; 45 negative cultures from field grown Refugee plants that developed secondary symptoms of mosaic. From field-grown mosaic Refugee plants diseased from seed 56 isolations yielded 27 cultures of micrococci distributed irregularly to the young seed. From rugose mosaic Refugee plants 56 isolations gave 18 cultures of micrococci, 13 cultures of Rickettsia, 21 negative cultures and 2 gross contaminations.

In a study of the progeny from 50 mosaic plants diseased from seed the distribution of mosaic in the seedlings seems to correspond with the irregular distribution of the micrococci to the various seeds of the pod. These studies suggest that the virus is localized in the plant and that its irregular distribution to the seed is due to this localization and to a vascular portal of entry to the ovules. A study of the vascular anatomy of the pod affords supporting evidence for this hypothesis.

The use of fertilizers in reducing losses from pea-root rot caused by Aphanomyces euteiches. C. M. HAENSELER.

The incidence of the pea root rot caused by *Aphanomyces euteiches* was delayed and the injurious effects on the host greatly reduced by the proper use of fertilizers.

Plots receiving 2,000 pounds per acre of a complete fertilizer gave 72.7 per cent increase in plant height and 111.5 per cent increase in yield. An application of 1,000 pounds per acre gave 41.8 per cent increase in size and 89.3 per cent increase in yield. The apparent severity of root rot on these plots by July 1 was rated zero for the 2,000-pound application, medium for the 1,000-pound plots, and very severe on the unfertilized plots. In another test, yield increases of 94 to 206 per cent on Aphanomyces-infested soils resulted from different fertilizer treatments. It was evident in all these tests that much of the increase in yield was due to retardation in development of the disease.

Greenhouse tests have shown that all the principal fertilizer salts tend to retard the development of the disease or decrease the percentage of infection. Nitrate of soda,

sulphate of ammonia, and muriate of potash were more effective in this respect than superphosphate.

Bottom rot of cabbage caused by Corticium vagum. G. F. WEBER.

A destructive and apparently undescribed disease of cabbage was observed in several fields in the vicinity of Gainesville, Florida, during the winter of 1930. The disease was most prevalent in low fields and in wet places in other fields. On individual plants the first symptoms are a browning and dying of the leaves at the basal portion of the head. If the plant is attacked early, the fungus grows rapidly into the head. If the head is older and compact, the fungus grows over the outer leaves, killing them. These leaves are detached and often shed or may remain, giving the head a brown and bald appearance.

The disease is apparently a further development of wire-stem, in which the fungus grows up the stem under humid conditions and attacks the more succulent tissue of the leaf blade. The fungus was observed in the fruiting stage on the host and it compared favorably with *Corticium vagum*. Inoculations made from pure cultures resulted in the reproduction of the disease on potted plants in the greenhouse.

Endohydrosis of forcing cucumbers and its control. RAY NELSON.

A disease of forcing cucumbers characterized by increased hydrostatic pressure, severe water-logging of the leaf tissues, bronzing of the leaf veins, and a general chlorotic and unthrifty condition of the plant has been prevalent in a 3-acre planting at Grand Rapids for the last 3 years. In 1929 the disease was responsible for a crop failure. The leaf spotting usually appears after the first pruning and is most severe during cloudy weather when transpiration is checked. The spotting of the leaves is due to the forcing of water into the intercellular spaces. Shortly after removal from the plant the leaf regains its normal appearance.

This disease apparently is associated with the use of excessive amounts of hen manure as a source of organic fertilizer. Heavy applications of this material are applied yearly to the soil which is then steam-sterilized for four hours. After the first pruning the leaf spotting increases up to the time the fruits begin to set, when this symptom becomes less pronounced. The plants soon become chlorotic and unproductive. Supplementary applications of mineral fertilizer, lacking nitrogen but high in phosphates and potash, have given satisfactory control.

Undescribed symptoms of mosaic in Porto Rico tobacco. MELVILLE T. COOK.

Small black spots resembling those caused by fungi have been observed on the old leaves of tobacco since 1923. No cause was discovered until the winter of 1929–30 when it was found that they occurred on the old leaves of mosaic plants and that they were formed in a very few hours and during the night. Mayer, in 1886, described spots of this kind, but of course it is impossible to say that they are the same. In 1890 Iwanowski and Poloffzoff declared that Mayer had confused two diseases and described these spots under the name of "pokenkrankheit."

These spots are as abundant in the green as in the chlorotic areas but the pattern usually disappears before they are formed. Cytological studies indicate a rapid disintegration of one or more palisade cells which spreads to the mesophyll. The final stage is the collapse of the epidermal cells.

Four apparently undescribed mosaics which go to tobacco. H. H. MCKINNEY.

1. A concentrated yellow mosaic is mild to medium-intense and produces a lacy type of mottling on tobacco. It does not go to tomato and tomato is not a carrier. It is medium-intense on *Nicotiana glauca*.

2. Another concentrated yellow mosaic is very intense and on tobacco and tomato it is indistinguishable from the yellow type previously described. On *N. glauca* the new type is very severe, whereas the previously described one is very mild or does not appear, and its virus is not completely systemic.

3. A green mosaic with a trace of a yellow type is practically indistinguishable from the common mosaic on tobacco. However, on *N. glauca* it is consistently more intense than the latter. All the above viruses are highly potent over long periods and have high thermal-destruction points.

4. Another green mosaic which is free of yellow is mild on tobacco. Mottling is most pronounced on the upper 10 leaves, fading later, causing characteristic premature yellowing on the older leaves. The mottling is unusually pronounced on tomato. It does not go to *N. glauca*. The potency of the virus is low and of short duration, being inactivated at comparatively low temperatures.

Epiphytology of tobacco mosaic in North Carolina. FREDERICK A. WOLF.

A study has been made during the past three seasons to determine by epiphytotic methods: (1) How true tobacco mosaic survives the winter in North Carolina; (2) by what agencies it is introduced into seed beds and into the fields; (3) by what agencies it is disseminated in the fields; (4) what factors govern its rate of spread and its severity; and (5) the applicability of methods of prevention and control. Observations were made on 465 seed beds, 85 of which were mosaic. Two hundred and twenty-nine fields were examined at intervals of approximately two weeks. At the beginning of harvest the average percentage of infection in these 229 fields was 22.7, approximately eight times as great as at the time when the plants had become established following transplanting. By the end of the harvest, the percentage of infection was approximately 95. The primary agent of introduction of the mosaic into seed beds and of dissemination in the fields is man, himself. Mosaic survives the winter, in fields, in the stubbles.

Further studies on virus purification. H. H. MCKINNEY.

Green mosaics from twenty-four sources have been studied on tobacco and related species. All but two of these contained traces of yellow mosaic when collected. In one mixed case the green type has been freed of the yellow type by successive dilutions and inoculations.

These yellow-free green mosaics are distinct from the common type occurring on tobacco and no difficulties have been encountered in keeping them free from yellow mosaic.

Virus of the common mosaic of tobacco has been subjected to dilution tests, chemical and physical treatments, and to tests on various hosts to eliminate the trace of yellow mosaic. All methods have failed and it is considered that we are dealing with a "virus complex."

Leaves from diseased plants grown near 27° C. with continuous light from Mazda lamps in soil of not too high nitrogen content yield virus extracts low in solids and soluble pigments and high in virus potency. The extraneous solids and soluble materials have been removed from these by centrifugation, combined with temperature coagulation, with less difficulty than is usual with extracts from plants grown under ordinary conditions.

Local lesions of mosaic in Nicotiana tabacum. FRANCIS O. HOLMES.

It has been commonly believed that leaves of *Nicotiana tabacum* never show local symptoms when inoculated with the virus of typical tobacco mosaic, and that symptoms appear only on leaves developing after inoculation. During the past three summers, however, yellowish local lesions have been observed and studied in green leaves of Turkish tobacco inoculated with tobacco-mosaic virus. These yellowish lesions proved to be sources of virus in high concentrations before near-by green areas furnished measurable amounts. The distribution of starch was abnormal in the affected areas. Leaves stained in iodine showed conspicuous patterns, which indicated the numbers and positions of points of entrance and multiplication of the virus.

Plastid pigment and chlorophyllase contents of tobacco plants as influenced by three types of mosaic. P. D. PETERSON.

The chlorophyll, carotin, and xanthophyll contents are lowered in tobacco leaves with mild dark green, light green, and intense yellow mosaics. The greatest reduction of all three pigments resulted from the yellow type and the least from the mild dark green type.

The chlorophyllase content of leaf tissues was greatly increased by the intense yellow mosaic in comparison with normal leaves. On the contrary, the chlorophyllase content of leaf tissue yellowed from age or nutritional disturbances was less than that of normal leaf tissue.

The chlorophyll content and chlorophyllase activity of normal leaf tissue seemed to be directly correlated, *i.e.*, the greater the chlorophyll content of the tissues, the greater the chlorophyllase activity. The reverse was true for mosaic-diseased leaf tissue; the light green or yellowed tissues, though lower in chlorophyll content, were higher in chlorophyllase than were the darker green leaf tissues.

There was a progressive decrease in both the chlorophyll content and the chlorophyllase activity of leaves from the top to the base of normal plants. Pith and root tissues were devoid of chlorophyll and evidenced little or no chlorophyllase activity.

Resistance of monocotyledonous plants to Phymatotrichum root rot. J. J. TAUBENHAUS and WALTER N. EZEKIEL.

Root rot caused by *Phymatotrichum omnivorum* attacks more than 500 cultivated and noncultivated species of plants. Monocotyledons have generally been considered resistant, although Phymatotrichum strands were sometimes observed on their roots when near other infected plants. King and Loomis, however, consider presence of strands as evidence that date palms, Johnson grass, Bermuda grass, and sorghum are hosts of root rot. Corn and cotton plants, in alternate hills, were inoculated with fresh cotton-root inoculum. Only the cotton plants succumbed to root rot. Depressed, deep brown lesions were found on roots of check and inoculated corn plants. Isolations from diseased cotton roots yielded typical Phymatotrichum growth. Isolations from the corn-root lesions and similar lesions on roots of sorghum, Johnson grass, corn, and other gramineous crops from root-rot-free or infested areas failed to yield any Phymatotrichum. Lesions of this sort are common in Texas on roots of gramineous plants. Other monocotyledons including canna, caladium, date palm, iris, tuberose, gladiolus, tiger lily, and nutgrass have been grown beside cotton plants and inoculated with root rot. Infection occurred only on the cotton. These experimental evidences suggest that monocotyledonous plants are not hosts to Phymatotrichum root rot.

Nutritional studies on Phymatotrichum omnivorum. WALTER N. EZEKIEL, J. J. TAUBENHAUS, and J. F. FUDGE.

Phymatotrichum omnivorum grows readily in synthetic media, even the sclerotial stage developing in cultures in which the source of nitrogen was ammonium nitrate and that of carbon dextrose. Heaviest growth was secured with a relatively large supply of dextrose and a lesser amount of some source of nitrogen (growth with ammonium nitrate was more than with peptone > urea > glycine > asparagin > potassium nitrate >leucine>ammonium sulphate). Phosphate was essential, also potassium or magnesium or possibly both. Iron, chlorine, and sulphate were omitted without significant change. Growth curves at 28–29° C. reached a peak in 5 weeks with a substratum high in dextrose and in 3 weeks with one of low dextrose content. The media became increasingly acid as colonies increased in weight but tended toward alkalinity, with subsequent degeneration of the mycelium.

In a single series, growth was approximately the same in a complete synthetic medium as with addition of autoclaved extracts from roots of corn (resistant to root rot) or of cotton (susceptible to root rot). Growth was slight but greater with cotton than with corn extracts diluted in water. In undiluted, ultrafiltered cotton-root extract growth appeared more successful than in a similar corn extract.

The resistance of Malvaviscus conzattii (arboreus) to Phymatotrichum root rot. W. J. BACH and J. J. TAUBENHAUS.

Plants of the Malvaceae family, such as cotton, okra, and the common Hibiscus (*Hibiscus rosae-sinensis* L.) are considered very susceptible to root rot caused by *Phymatotrichum omnivorum*. On the other hand, *Malvaviscus conzattii* (*arboreus*), commonly known as the Turk's-cap Hibiscus, one of the Malvaceae, appears to be an exception. This perennial, woody shrub is grown extensively in the lower Rio Grande Valley of Texas as an ornamental. It has been under observation for the past eight years, and, although continually growing adjacent to or mixed with other susceptible hosts, was not found dying from Phymatotrichum root rot.

Repeated artificial inoculations of *Malvaviscus conzattii* (*arboreus*) with pure cultures or with the method reported elsewhere, failed to kill a single well-established plant. Work is now in progress to determine whether resistance is due to possible morphological differences or to some physiological nature inherent in the host.

Development of root rot in cotton planted at different dates. B. F. DANA and H. E. REA.

At Texas Substation No. 5, Temple, Texas, during the 1928 season seven different cotton varieties, ranging from early to late, were each planted at seven dates: March 15, April 1 and 15, May 1 and 15, and June 1 and 15. Root rot appeared at about the same time and increased at nearly the same rate in cotton of the first four planting dates with no differences attributable to varieties. Root rot was delayed in appearance in the June plantings. The total amount of disease at the end of the season was appreciably lessened only in the June 15 plantings. These results indicate that factors other than earliness in the crop govern the time of appearance and rate of development of root rot in cotton. The experiment was repeated in 1929 and 1930 with three varieties planted at three dates. While the disease was less in late-season plantings, yields were unsatisfactory due to drought and insects. It appears that factors favorable for the cotton crop are also favorable for the disease, making it difficult to manipulate the planting date in the control of the root-rot disease.

Cultural and inoculation experiments with Taphrina Potentillae. ELLA M. MARTIN.

During the summer of 1928, a number of cultures of *Taphrina Potentillae* were isolated from plants of *Potentilla canadensis* growing in the vicinity of Ithaca, New York. This organism causes leaf spot of *P. canadensis.* Cultures of *T. Potentillae* on potato-dextrose agar are pale pink, glistening and opaque, and in appearance are very similar to those of *T. Johansonii, T. mirabilis, T. coerulescens,* and *T. deformans.* Cultures of *T. Potentillae* were brought to Greensboro, North Carolina, where they were used in inoculating plants of *P. canadensis.* Inoculation of 38 plants out of doors in March, 1929, and of 16 plants out of doors in March, 1930, produced respectively 50 per cent and 75 per cent of infection. A number of other plants of *P. canadensis* were inoculated with the same cultures in October and November, 1928 and 1929, but no infection resulted, though some of these plants were grown all winter out of doors under cheesecloth and others were grown in the laboratory.

Cytological studies of *Taphrina Potentillae* showed that the development and budding of the ascospores are similar to those reported for *T. coryli* and other species of Taphrina. Ascospores of *Taphrina Potentillae* were found to conjugate in drops of potato-dextrose agar, Sabouraud's agar, decoction of Potentilla leaves, and dextrose agar made with Potentilla leaves.

Progress of Fusarium wilt inside the rhizomes of banana plants. F. L. WELLMAN.

True stems of Gros Michel bananas are bulbous rhizomes with short, horizontally borne, underground stolons. Branches have terminal buds which produce the aerial pseudostem, leaves, and stalk of fruit. Clumps or "mats" of connected plants come from a seed bit cut from a rhizome. *Fusarium cubense* is a vascular parasite producing distinct, readily recognizable, discolored water-conducting tissues. A banana mat becomes infected through uninjured roots or wounds in rhizomes and the disease spreads from plant to plant through underground connections. In dissected rhizomes it is possible to trace the course of the disease many months after infection. It spreads most rapidly inside the rhizome stele, occurring typically at first on one side next to the endodermis. It progresses most easily towards the growing point of the rhizome and from plant to plant in the mat through six generations before symptoms appear in the plant above ground. Buds on the rhizome side affected by the disease are stunted but the undifferentiated tissues are not involved except in very extreme cases. Individuals were found in which Fusarium was recovered in pure culture from roots, rhizome, and leaf sheaths, but not in the fruit stalk attached.

Corm treatments for gladiolus and calla lily. PAUL E. TILFORD.

Various formaldehyde and mercury treatments have been tried on gladiolus corms in an attempt to find a simple and effective measure for controlling scab (*Bacterium marginatum*). The standard treatment, soaking for 2 hours in $HgCl_2$, 1–1000, is difficult when large quantities of many varieties are treated.

Formaldehyde dusts and hot formaldehyde treatments proved ineffective and retarded flower and corm production. Acidulated $HgCl_2$, 1–500, treatments for 5, 10, and 15 minutes were ineffective. Calomel suspension, 1 pound to 2½ gallons of water proved very effective. Semesan solution, 1 per cent, for 7 hours, controlled scab and stimulated corm production but caused plants to flower later. Organic mercury compounds containing ethyl mercury chloride proved very injurious, a high percentage of the corms were killed, flowering delayed several weeks, and scab was not satisfactorily controlled. Bayer-Semesan compound 694 appears to have possibilities as a short, effective treatment.

The Phytophthora root rot of the white calla lily has been very destructive in Ohio for the last 2 years. Extensive corm-treatment experiments are under way and preliminary results are promising.

Powdery mildew of red clover. CECIL YARWOOD.

The powdery mildew of red clover, *Erysiphe polygoni*, is readily carried in culture on excised red-clover leaflets removed in the late afternoon and floated on a 6 per cent sucrose solution in Syracuse watch glasses. Growth of the parasite is favored by an abundant carbohydrate content of the leaflet. The incubation period is about 6 days. In field plots of equally spaced clover plants of all degrees of susceptibility, a correlation was established between mildew severity and yield. A wide range of resistance and susceptibility was found in seed from the same parent. Two physiologic forms of red-clover mildew have been separated which differ very markedly in their reactions on certain individual plants. As high as 97 per cent germination of spores has been observed. The spores are relatively resistant to the action of certain chemicals but sensitive to sulphur. Germination may occur in a relatively dry atmosphere, although some of the spores and germ tubes may collapse after germination. The germ tubes are negatively phototropic. Spores showing little or no germination can sometimes be stimulated with sucrose solutions to relatively high germination. On the leaflets of a resistant host, reduced germination often occurs.

(Cooperative investigations between the Office of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Botany Department, Purdue University Agricultural Experiment Station.)

The comparative value of checking and drilling in the control of Cercospora leaf spot and yield of sugar beets. I. E. MELHUS and EDGAR F. VESTAL.

Experiments conducted in the field have shown that Cercospora leaf-spot infection is markedly influenced by humidity. Sugar beets are normally thinned to 12 inches in the row, but by increasing this spacing to 20 inches in checked beets the humidity was reduced from 3 to 10 per cent, soil moisture increased about 1 per cent, evaporation increased 3.6 cc. per day, and the wind velocity increased 10 feet per minute over the normally spaced beets. These factors all tend to decrease leaf spot and to increase yield, as is evidenced by the fact that normally thinned beets contained six times as many naturally occurring infection centers and 1,960 pounds of beets per acre less than the checked beets. Analyses of sugar beets from both types of thinning showed a purity of 82.5 and a sugar percentage of 14.56 for the checked, compared to 80.5 and 15.61 for the drilled. However, the increased tonnage of the checked beets more than offset the increased sugar percentage of the drilled.

Checked beets, thinned to one double every other place, and every third place, yielded 33,184 and 33,679 pounds per acre, respectively, as compared to 32,788 and 29,745 pounds per acre, respectively, for checked beets thinned to one to a place and normally drilled beets. Doubles did not reduce the yield.

Alfalfa mosaic.—J. L. WEIMER.

The occurrence of a mosaic of alfalfa has been reported on different occasions to the Plant Disease Survey, U. S. Department of Agriculture. It is believed that these reports have been the records of field observations only. So far as the writer is aware, the existence of a transmissible mosaic of alfalfa heretofore has never been proved experimentally. A mosaic disease of this plant occurs commonly in California and has

been shown to be readily transmissible by aphids (*Illinoia pisi*). Thus far efforts to transmit it mechanically have failed. Evidently the same disease has been found in Wisconsin recently by F. R. Jones who sent affected plants to the writer for comparison with California material. The disease is most prevalent during the cooler parts of the year and is especially abundant in the spring before the first cutting. The losses caused by this disease are very small. So far as observed, affected plants are never killed, the damage being limited largely to a slight amount of dwarfing in the most seriously affected plants. The host range of the virus causing this disease is yet unknown. (Cooperative investigations between the Office of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the California Agricultural Experiment Station.)

Varietal susceptibility, distribution, and control of yellow dwarf of onions. W. J. HENDERSON.

Yellow dwarf of onions is a transmissible virus disease overwintering in the sets and mother bulbs. Field tests in 1929 and 1930 on 35 onion varieties show that the Sweet Spanish varieties possess a high degree of tolerance but are not suited to Iowa conditions. This disease apparently is not localized in Iowa. Inoculations from specimens collected by N. J. Giddings show that it occurs in West Virginia. Giddings has supplied photographs taken in 1916 of what appears to be yellow dwarf. In the autumn of 1929, L. D. Leach sent specimens from California which proved to contain the yellow-dwarf virus.

Indexing onion sets and isolating the set plots have reduced the amount of yellow dwarf in the Pleasant Valley district from 45 per cent in 1928 and 20 per cent in 1929 to 1 per cent in 1930. It is believed that this disease is entirely amenable to control by the methods utilized.

The greenhouse method of indexing sets is being replaced by a water-culture method, in which the sets and mother bulbs are grown on a coarse wire screen resting on the surface of a dilute nutrient solution. This method indicates the amount of infection as well as the greenhouse method and can be carried out under ordinary home conditions.

Virulence of attenuated curly-top virus restored by Stellaria media. C. F. LACKEY.

The virulent form of curly-top virus, attenuated by passage through nettle-leafed goosefoot, *Chenopodium murale*, is restored to approximately its original virulence by passage through chickweed, *Stellaria media*. The following results are representative of those which demonstrate this fact:

Kind of virus	Number of beets inoculated	Per cent diseased	Average incubation period in days	Severity of symptoms
Original (virulent)	65	64	9.5	Severe
Attenuated	83	25	13.1	Very mild
Restored	110	74	9.9	Severe

The symptoms produced by the restored virus were indistinguishable from those produced by the original, virulent virus. These consisted of severe dwarfing and distortion of the leaves. The attenuated virus by contrast produced very slight dwarfing and distortion.

Chickweed is probably not an important factor in restoring the virulence of the virus in nature because it grows in moist and shady places which are unfavorable for the beet leaf hopper, *Eutettix tenellus*, the vector of the virus.

Filaree, *Erodium cicutarium*, however, is a very important host for the leaf hopper in California during winter and early spring. It also plays an important rôle in the overwintering of the virus. Investigations are now in progress to determine its effect on the attenuated virus.

New virus diseases in Porto Rico. MELVILLE T. COOK.

Brief descriptions of six unreported virus diseases follow. A mosaic of *Crotolaria striata* dwarfs the plant and reduces seed production, but it is not carried in the seed. A rare mosaic of *Commelina longicaulis* appears only when the plant is making a vigorous growth and does not injure the plant. A bunchy top of *Papaya carica* appears to be due to a virus. It is very destructive but easily eradicated. A variegation of *Abutilon hirtum* is quite common. It dwarfs the plant to some extent and reduces seed production, but it is not carried in the seed. A variegation of several species of Sida may be due to the same virus. A mottling of the leaves of several species of mulberry appears to be due to a virus. It can be transmitted by scions.

The effect of ultra-violet radiation upon representative species of Fusarium. ALICE A. BAILEY.

Ultra-violet radiation has been found effective in increasing the amount of sporulation and the percentage of macrospores produced in the various species of Fusarium causing bulb rot of onions. Strains which usually produce mostly microspores in artificial culture produce abundant normal macrospores after radiation. In the case of a strain that had never produced any spores in culture during the 4 years succeeding isolation from a decayed bulb, macrospores were produced in abundance after radiation.

Because of its value in the work on taxonomy of onion Fusaria, radiation has been tried on some 25 species of Fusarium, representing all but one of the sections, in the hope that macrospores could be produced in such species as ordinarily produce only microspores in pure culture. In most instances radiation increased the total number of spores and the percentage of macrospores, where macrospores are characteristic of the species. In some few cases radiation reduced the number of spores. Some filters are more effective than others in screening out rays of detrimental wave length and permitting advantageous rays to pass through.

Plant extracts and fungi. II. Bean extracts in relation to Colletotrichum lindemuthianum. E. S. REYNOLDS and B. S. MILLER.

Extracts were made from bean plants which had been dried artificially at about 80° C., and then ground to a fine powder. An amount of water equivalent to the water content of the growing plants was used and mineral salts and glucose were added. Liquid cultures made with these undiluted extracts from certain varieties of young bean plants have been found to prevent growth of *Colletotrichum lindemuthianum*. Agar plates made with similar extracts supported a slowly developing growth of the fungus. Check cultures, having minerals and glucose in the same proportions as used in the bean-extract cultures, produced a good growth of the fungus. Preliminary studies indicate varietal differences in quantity of toxic action.

A destructive fungous disease of the corn borer. C. L. LEFEBVRE.

During the past year an epidemic of *Beauveria Bassiana* on larvae of the corn borer was observed in the European Corn Borer Laboratories at Arlington, Massachusetts, the

mortality among the larvae arising as high as 90 per cent in lots imported from Manchuria.

The occurrence of *B. Bassiana*, to the writer's knowledge, has never been reported on the corn borer in the United States. In laboratory experiments, 100 per cent larval mortality is obtained within two days when inoculated with conidia of *B. Bassiana*, while in experiments with *B. globulifera*, which is common in the United States, only 4 larvae were killed in seven trials of 10 larvae, each. Preliminary field tests were made, in which spores of *B. Bassiana* were dusted on corn-borer-infested fields. These trials indicated that at least a partial control of the corn borer can be obtained. The disease is first noted by the infected larvae turning pink from which the malady gets its name the *Pink Disease*. The larvae soon become mummified, and after a few days a white mycelial outgrowth is evident, which turns to a creamy, powdery mass, due to abundant spore formation.

B. Bassiana on artificial media is characterized by a flat, mealy, pulverulent growth, forming conidia in abundance within several days. *B. globulifera* on artificial media produces an elevated, cottony, floccose growth, not forming conidia for several weeks. In Van Tieghem cells, spores of *B. globulifera* produce much more extensive germ tubes which branch profusely throughout the droplet as compared to *B. Bassiana*. On artificial media, *B. Bassiana* does not readily lose its virulence.

Sclerospora butleri, a new species from Nyasaland. W. H. WESTON, JR.

The fungus was found on the wild grass, *Eragrostis aspera*, among tobacco plantings at Bulaki, Nyasaland, South East Africa, by E. J. Butler. It is characterized by the fact that the oospores (17 to 25 μ diam.) are smaller than any yet known, while the surface of the dark amber oogonial wall instead of being roughly patchy or ridged, angled and polygonally-faced as in other species, is marked by pallid, rounded protrusions 2 to 3 μ broad and 2 to 5 μ high.

The host shows the well-known shredding of the leaves which are infested by the resting spores only. The conidial phase is being sought to determine whether it produces zoosporangia germinating by zoospores as in typical *Sclerospora graminicola* or conidia germinating by hyphae as in other species.

As yet the fungus is known only from this collection on this grass, but the host has a wide range, occurring throughout Africa even to elevations of 2,000 meters and extending into southern India.

The Dutch elm disease in Ohio. CURTIS MAY, O. N. LIMING, and THELMA ALEXANDER.

Five cases of the Dutch elm disease were found on American elm in Ohio during the past summer. Symptoms were a wilting of leaves and new growth followed sometimes by yellowing and defoliation and at other times by browning of leaves. One or more annual rings showed a spotted brownish discoloration. Verticillium wilt of elm, because of similar field symptoms, can be distinguished from the Dutch elm disease only by cultures.

The fungus, isolated from roots, trunks, and twigs was culturally and morphologically similar to that described for *Graphium ulmi*. Buisman confirmed this identification, and Koch's rules of proof were satisfied.

In culture the colonies enlarged most rapidly at pH 4.8–5.9 and from 19°–27° C. They were yeast-like at pH 7.4–8.4.

Mycelium of the fungus was found abundantly in the tracheae, wood parenchyma, fibers, and wood rays of inoculated trees, but in naturally infected trees it was not common and was found only in the tracheae. It is intra-cellular and apparently passes from cell to cell through the numerous pits.

New methods for determining rate of decay behind cavity fillings in trees. W. HOWARD RANKIN.

Cavity methods should be judged by the subsequent rate of decay because complete eradication of the fungus is probably rarely accomplished and often unwarranted. Wound decays, naturally sealed in by callus growth, are known to be arrested. An effective cavity treatment should accomplish the same result if it seals the opening. Initiating decay in small cavities dug in sound wood by imbedding blocks of wound-decay material has been attempted in an extensive series in oaks and maples. To study the fundamental factors, apparatus has been installed in filled cavities which makes possible the insertion and withdrawal of pure-culture material without disturbing the filling or callus. This apparatus also provides for the analysis of the gases in the wood. The carbon dioxide content of trees and of the wood behind various types of fillings is being studied as the possible factor accounting for the cessation of decay in closed wounds. The X-rays have been used successfully to show accurately slight amounts of decay in living trees. Radiographs provide a means of annually recording the rate of decay behind fillings of different types not only in experimental cavities but also in those of unknown previous history.

Use of oxides of unsaturated hydrocarbons for the eradication of barberries and other pests. R. B. HARVEY.

Practical applications have been made of ethylene oxide and propylene oxide for killing barberries, gooseberries, and other noxious plants. Ethylene oxide diffuses well in soil and into the plant, producing death in one or two weeks. The stem tissues blacken and leaves fall off, indicating death of the plants. A "depth charge" is injected by means of prod rod provided with a measuring chamber. With this "gopher stick" a measured charge is injected into the soil beneath the plant, using the pressure of the ethylene oxide directly from the tank, or a solution of ethylene oxide in water can be injected from the usual knapsack sprayer.

Corynose twig blight of the American bladder nut. W. H. DAVIS.

During 1929–30, a severe twig blight of the American bladder nut, *Staphylea trifolia* L., was observed in Massachusetts. Some of the smaller shrubs were killed while half the current year's growth of others was destroyed.

During April, 1930, monosporous cultures of the associated fungus were made and incubated on steamed oat-meal, steamed yellow corn-meal and potato-dextrose agars. Conidia and mycelium from these cultures were employed in making inoculations during May and July. Infections showed that the fungus was pathogenic and hyphae entered through meristematic tissues of twigs at the tips, nodes, or leaf axils.

The fungus somewhat resembles *Coryneum microstictum.* Conidia were sub-pyriform; 6 x 18 μ (Saccardo; 5–6.5 x 15–17 μ); apex obtuse, 4 loculi, lowest loculus subhyaline; those above, honey-color; conidiophores, filiform, hyaline. However, these differences were noted; stroma present, not absent or obsolete; conidiophores, 1.3 x 19, not 1.5 x 20–25 μ. *Staphylea trifolia* is an unreported host. The classification suggested for the fungus is: *Coryneum microstictum* B. and Br. variety, staphyleae.

A survey for stinking smut in wheat. R. J. HASKELL, R. C. ROSE, W. E. BRENTZEL, E. A. WALKER, and WALDO KIDDER.

An examination of 814 wheat fields comprising 66,729 acres in 17 counties of Minnesota, the Dakotas, and Montana, in 1930, has shown that 62 per cent of the spring- and 92 per cent of the winter-wheat growers treated their seed. The average percentage of

smut was: spring wheat treated 2, untreated 4, total 2.8; winter wheat (110 fields in Montana) treated 5.9, untreated 24.8, total 7.4.

Formaldehyde was used on 74 per cent of the spring and 25 per cent of the winter wheat; copper carbonate on 20 per cent of the spring and 55 per cent of the winter.

The percentages of smut in spring wheat following different treatments were: 50 per cent copper carbonate with machine, 0.3; 20 per cent copper carbonate, commercial machine, 0.5; formaldehyde, machine, 0.9; 20 per cent copper carbonate, home made machine, 1.1; Ceresan, machine, 1.3; Ceresan, shoveled, 1.3; formaldehyde, dip, 2.0; formaldehyde, sprinkle, 2.1; 50 per cent copper carbonate, shoveled, 3.2; 20 per cent copper carbonate, shoveled, 4.6. Winter wheat results were comparable. Spring wheat treated annually had 1.4 per cent. smut; not treated annually, 4.7 per cent. The leading spring wheats showed the following percentages of smut in untreated fields, Ceres 9, durum 7, Marquis 4, Ruby 2, Marquillo trace, all hard red spring wheats 2.7, all durums 2.9.

Effect of time and rate of application of seed disinfectants on oats and wheat. BENJAMIN KOEHLER.

Tests were conducted for 2 years with formaldehyde dust (Smuttox in 1929, Corona Oat Dust in 1930) on 2 varieties of oats. Treatments made 3 months, one month, and one week before seeding all caused reductions in yield of grain as compared with treatments made one day before seeding, the depression being in proportion to length of time. Treatments made with Ceresan 3 months or one month before seeding also caused some depressions in yield.

On smutty seed, all these treatments, however, caused increases in yield ranging from 0.7 to 10.7 bushels for formaldehyde dust and 3.9 to 14.9 for Ceresan, according to time of application. On seed nearly free from smut the long-time treatments caused a loss in yield. Three ounces per bushel controlled oat smut better than 2 ounces.

Hred wheat was treated with copper carbonate, extended copper carbonate, Ceresan, and other materials during the last 2 years. The seed contained some Gibberella infection both years, but no bunt infestation. Significant increases in yield ranging from 2.4 to 11.7 bushels per acre were obtained with the compounds named. Two ounces per bushel of any one of them proved just as effective as 3 ounces.

Wheat diseases in Tennessee. C. D. SHERBAKOFF.

Sulphur dusting for the prevention of a black chaff of wheat. F. J. GREANEY.

In testing the effectiveness of sulphur in controlling leaf rust *Puccinia triticina* Eriks., and some of the minor leaf and stem diseases of wheat, it was found that frequent applications of sulphur dust prevented the development of a bacterial disease of wheat, called black chaff.

At harvest time the percentage of nondusted wheat plants infected with black chaff ranged from 65 to 95, with an average of 75 per cent; whereas the range of the dusted plants was from a trace to 8 per cent, with an average of 4 per cent. In the absence of significant amounts of leaf and stem rust the statistically significant increased yield of 5.9 bushels per acre resulting from dusting was due in a large part to the control of black chaff.

The results of the sulphur-dusting experiment suggest very strongly that the organism causing black chaff spreads from plant to plant in the field. Wind and rain probably are the most important agents of dissemination. This disease has not been positively identified as the black-chaff disease caused by *Bacterium translucens* var. *undulosum*, but in general symptoms they are identical.

Cold injury. J. R. HOLBERT and W. L. BURLISON.

Progeny studies, in the field, of yellow dent corn plants grown from mature seed unexposed and exposed ten hours to three different temperatures, 32, 23, and 14°F., respectively, in field refrigeration chambers in the fall of 1929, indicate that seed value of some strains of corn, with 20 per cent moisture in the grain, was injured by exposure to temperatures of 32° F. and lower. However, there were marked differences between different inbred and crossbred strains. Germination studies and field yields indicate that germination does not always give reliable index of extent of cold injury.

As determined by temperature measurements by thermoelectrical methods, different corresponding tissues of corn plants of different strains differed greatly in rates at which they cooled when exposed to chilling and subfreezing temperatures in refrigeration chambers. Husks of some strains offered more protection to shanks and grain than did husks of other strains.

Application of phosphate to soil prior to planting greatly increased cold resistance of young corn plants of both cold resistant and cold susceptible strains.

Three varieties of soybeans, Wilson, Virginia, and Illini, differed greatly in their resistance and susceptibility to cold injury in the late maturation stages. (Cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Illinois Agricultural Experiment Station, and Funk Bros. Seed Company.)

Relation of seed quality to yielding ability and disease resistance in hybrid strains of dent corn. R. R. ST. JOHN and J. F. TROST.

Comparisons were made of the field performance of crossed seed selected from healthy inbred or crossbred plants with similar seed selected from corresponding diseased plants, all grown under conditions of continuous corn culture, on similar soil, with an abundance of natural inoculum of the various root-, stalk-, and ear-rot fungi. These seed quality comparisons involved 37 distinct hybrid combinations of dent corn. Weather conditions favored abundant stalk and ear infection by *Diplodia zeae*.

As a grand average for all 37 hybrids, plants from seed from healthy plants yielded 4 per cent more than the plants from seed from diseased plants. In paired comparisons, the seed from healthy plants was superior 84 out of the possible 111 times.

There was no significant difference in the percentage of nubbins, broken stalks, or ear rot caused by *Diplodia zeae*.

The very best crossed seed of hybrid strains that were either too early in maturity or were still immature at the time of killing frosts did not yield so well as ordinary low-quality crossed seed from diseased plants of those hybrid strains that matured by the time of killing frosts. (Cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Botany Department, Purdue University Agricultural Experiment Station.)

Resistance in sweet corn to Diplodia zeae. GLENN M. SMITH and JOHN F. TROST.

In 1930, field trials of inbred and crossbred strains of sweet corn were conducted on land that had been in continuous corn culture for ascertaining the relative resistance to natural infection by the various root-, stalk-, and ear-rot fungi. A comparison of the amount of Diplodia infection in sweet corn and in dent corn grown in the same field showed 7.5 per cent ear infection for 500 strains of dent corn and 10 per cent ear infection as an average of 225 strains of sweet corn. Apparently, sweet corn is inherently as resistant to ear rotting by *Diplodia zeae* as is dent corn.

In these experiments 65 strains of sweet corn showed less than 5 per cent ear infection by *Diplodia zeae*, while 23 strains showed more than 30 per cent.

Inbred lines, selected solely on the basis of horticultural performance during the first five years of their inbreeding, had a significantly higher percentage of ear infection than those inbred lines selected for disease resistance throughout their inbreeding.

In a number of instances where both parents of single-cross hybrids were highly susceptible to ear infection, the hybrids were nearly free from such infections. (Cooperative investigations by Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Botany Department, Purdue University Agricultural Experiment Station.)

Cornstalk rot and ear rot. A. L. SMITH and J. R. HOLBERT.

Stalk and shank tissues of nearly mature corn plants injured by exposure to low temperatures in field refrigeration chambers have been found to be much more susceptible to infection from artificial inoculation with *Basisporium gallarum* than comparable tissues of corn plants not injured by cold. Likewise, stalk and shank tissues of corn plants grown on old soil were more susceptible than were comparable plants grown on new soil.

Marked differences in relative resistance of stalk and shank tissues to infection from artificial inoculation with *Diplodia zeae* were found in both inbred and crossbred strains of corn.

Stalks of corn plants grown on more productive soils were more resistant to natural infection from *D. zeae* than stalks of comparable plants grown on less productive soil. (Cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Wisconsin Agricultural Experiment Station, and Funk Bros. Seed Company.)

Nodal infection with the corn-smut organism. I. E. MELHUS and GLEN N. DAVIS.

When 500 sweet-corn plants, variety Golden Bantam, one foot tall, were inoculated with two opposite monosporidial suspensions in carrot decoction, having a surface tension of 47 dynes, 33 per cent of the plants became infected. When the surface tension was changed to 34.4 dynes, by the addition of fish-oil soap, 91 per cent became infected. No smut boils developed on the lower nodes in the former case, while 40 per cent of the infected plants in the latter produced smut boils; and 54.5 per cent of the smut boils produced did not express themselves until after the plants had tasseled.

When three inbred lines, reputed as very resistant, showing an average of only 1.4 per cent natural infection in the field, were inoculated with a low surface-tension spore suspension, boils were produced on 35 per cent of the plants.

In both field and greenhouse experiments, when the leaf sheaths were removed late in the development of the plant, many were found where infected axillary buds existed with little hypertrophied tissue. It is believed that all axillary bud infection resulted from spores dropping into the spiral whorl and manifestations of the infections were only in those buds which became active through any cause.

Basisporium dry rot of dent corn as related to temperature and cob reaction. C. S. REDDY.

From many experiments in which Basisporium-infected dent corn seed was germinated at different temperatures and in which different strains of corn were germinated at the same temperature, it was apparent that severe injury occurred in the form of seed rotting when the temperature relation to the seed prevented early germination. No apparent injury occurred immediately after germination; therefore, there is no seedling-blight stage.

Susceptibility to Basisporium ear rot was directly correlated with low acidity and resistance with high, as determined in distilled water extracts of disease-free cobs.

Cob pH values	Inbreds in class interval	Ears observed	Ears infected
	No.	No.	Per cent
4.4–4.7	5	116	0
4.8	6	121	2.5
4.9–5.0	14	312	7.4
5.1–5.2	16	313	22.7
5.3–5.4	12	258	38.0
5.5–5.6	7	175	41.7
5.7–5.8	8	185	33.5
5.9–6.3	7	173	48.6

These two tests are especially suitable for use by corn breeders, because, in their application, they require no knowledge of plant diseases and may be used every year whether or not there is an epidemic of Basisporium ear rot.

Fifth progress report on studies of fall applications of fungicides in relation to apple-scab control. G. W. KEITT.

Penetration and toxicities of petroleum-oil sprays. P. A. YOUNG.

Apple and potato leaves partly soaked with pure oils (6 per cent or less sulphonatable residues) lived for 30 to 80 days. Pure oils with more than 13 per cent sulphonatable residues killed apple leaves within 7 to 14 days; they were toxic to apple and potato leaves in 4 to 8 per cent oil sprays, being much more injurious than nearly saturated oils. Winter killing was associated with abundant oil in apple twigs. Oils spread between parenchyma cells and in tracheae of apple limbs, leaves, and fruit. One per cent sprays with nearly saturated oils caused no macroscopic symptoms in apple or potato leaves; eight per cent sprays caused some black sports in potato leaves. Oils spread mainly between parenchyma cells in potato and cucumber stems. Oils passed from potato leaves into the tubers. Protoplasm flowed for eight hours in staminal hairs of *Tradescantia fluminensis* immersed in pure, nearly saturated oils. *Helminthosporium sativum*, *Rhizopus nigricans*, *Achlya conspicua*, *Glomerula repens*, and *Penicillium* sp. grew abundantly and anaerobically in pure oils. They grew best in nearly saturated oils. Earthworms (Annelida) and larvae of *Phorbia brassicae* lived for a few hours in pure, nearly saturated oils but not in less saturated oils.

The relation of pentathionic acid and its constituents to the toxicity of sulphur fungicides. O. NEAL LIMING.

Since 1928 a further study has been made of the chemical and physical nature and the toxicity of pentathionic acid and of its constituents, sulphur, sulphur dioxide, and hydrogen sulphide. Although the toxicity of sulphur to fungi has been attributed to a number of factors, only volatilized sulphur, sulphur dioxide, hydrogen sulphide, and pentathionic acid are now usually considered.

The volatile product of sulphur is a vapor. At summer temperatures, over ten per cent of the sulphur on a dusted surface may pass off as a vapor within two weeks.

Sulphur vapor is not toxic to fungus spores, and the condensation products are toxic only after standing several hours. Sulphur dioxide occurs only in traces in ground sulphur and is not toxic in such concentrations. Hydrogen sulphide does not occur in ground sulphur. Traces of this gas are produced from sulphur when in contact with higher plants and fungi, but it is not toxic to fungi in such conentrations. Pentathionic acid is associated with ground sulphur and in concentrations toxic to fungus spores. It is a natural oxidation product of sulphur but its formation is enhanced by mild oxidizing agents and possibly by hydrogen sulphide. It is not sufficiently volatile in dilute solutions to be toxic at a distance. The pentathionate ion is stable in acid and weak alkaline solutions but is toxic only in acid solutions. The toxic action is governed by the condition of the fungus rather than by the condition of the pentathionate ion.

Water-soluble arsenic in spray materials. H. C. YOUNG.

When lime sulphur and lead arsenate are mixed together in the proportion generally used for spraying a black sludge primarily composed of lead sulphide is produced. As lead sulphide is formed a portion of the arsenic becomes free. It was found that hydrogen sulphide normally present in lime sulphur is primarily responsible for the breaking down of lead arsenate and that this reaction is accelerated as the line sulphur is diluted. The arsenical determinations were made on spray materials dried on rubber mats at constant humidity and temperature. An attempt was made to duplicate actual spraying conditions and to obtain results comparable to actual field spraying. The water-soluble arsenic ranged from 6 per cent in the 1–40 lime sulphur dilution to 12 per cent in the 1–80 dilution.

An attempt was next made to add materials that would check the above reaction. It was found that calcium hydrate was the only effective one out of the following list: calcium carbonate, calcium chloride, ferrous sulphate, aluminum sulphate, aluminum hydrate, calcium caseinate, zinc hydrate, barium hydrate and carbonate and magnesium limes.

A mixture of calcium monosulphide and arsenate of lead resulted in a high percentage of free arsenic. Other fungicides mixed with arsenate of lead, including Dry mix, Sulfuron, Mulsoid sulphur, and Mist Brand wettable sulphur were also tested.

Results with new sulphur dusts for apple-scab control. A. L. PIERSTORFF and H. C. YOUNG.

The failure of most sulphur dusts to control apple scab during seasons favorable for the development of the fungus is attributed to their failure to adhere evenly to the foliage and fruit surfaces, and to insufficient toxicity.

In an attempt to overcome these difficulties two new dusts have been developed; namely, 85–15 sulphur—dry lime sulphur and 85–10–5 sulphur—manganar—aluminum-hydrate. These two dusts adhere to the foliage better and are more toxic to the apple-scab fungus than ordinary dusting sulphur. In three years' tests, two of which have been severe scab seasons, these dusts produced in 7 orchards 92.3 per cent clean fruit for the sulphur—dry lime sulphur and 85.7 per cent clean fruit for the sulphur—manganar—aluminum hydrate, while the plots dusted with a commercial-sulphur dust had 71.6 per cent clean fruit. The checks averaged 14.5 per cent clean fruit. The same variety was used for all plots. The dusts were applied throughout the season with the exception of a delayed dormant spray.

Hydrogen sulphide as related to the fungicidal action of sulphur. S. E. A. McCALLAN and FRANK WILCOXON.

Hydrogen sulphide is exceedingly toxic to fungus spores and is evolved when sulphur is applied to the surface of spores and leaves of higher plants.

The germination of conidia of *Venturia inaequalis*, of uredospores of *Uromyces caryophyllinus* and *Puccinia antirrhini*, and of conidia of *Sclerotinia americana*, *Macrosporium sarcinaeforme*, *Pestalotia stellata*, *Glomerella cingulata*, and *Botrytis cinerea* is completely inhibited at hydrogen sulphide concentrations ranging, respectively, from 0.2 to 40 milligrams per liter of solution. This is also the identical order of sensitivity of these fungi towards sulphur.

The evolution of hydrogen sulphide has been demonstrated from all species tested—19 fungi and 26 higher plants. The amounts produced by known quantities of spores and by strawberry plants have been determined. The production by spores appears dependent on the quantity of spores, increases with temperature to about 30° C., ceasing at 65° C., and occurs over a pH range from 4.0 to 8.0 with no well-defined optimum. Spores of Sclerotinia and Glomerella produce, respectively, 5 and 15 per cent of their weight of hydrogen sulphide in six hours.

Indications point to the reduction of sulphur on or within the spores and its initiation by glutathione. Glutathione has been demonstrated in spores of Sclerotinia.